

Graduate School for Cellular and Biomedical Sciences

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Development of new sedation protocols allowing improved and safer standing sedation of horses with a reduced risk for persons involved. Advantages of the addition of butorphanol.

PhD Thesis submitted by

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1. Abstract

Due to the high risk associated with equine anaesthesia, whenever possible diagnostic and minor surgical procedures are performed on the standing sedated horse. However a reliable sedation protocol is essential. The protocol should provide sufficient depth and duration of sedation, good analgesia, no or minimal reaction to external stimuli, and produce minimal ataxia and cardiovascular side effects.

Four trials were conducted in experimental horses in order to find an ideal sedation protocol for prolonged sedation. Two α_2 -adrenergic agonists (xylazine and romifidine) both registered for horses were studied with and without the adjunct of the opioid butorphanol. Constant rate infusion protocols providing stable sedation and constant plasma concentrations were developed for both α_2 -adrenergic agonists during the first two trials. The addition of butorphanol did not decrease α_2 -adrenergic agonist requirements and produced dangerous postural instability as well as inconsistent sedation when combined with xylazine. Therefore butorphanol was excluded from further trials. The xylazine and romifidine protocols developed during trial one and two were compared for degree of sedation, postural instability and reaction to different stimuli during the third trial and regarding influence on cardiopulmonary function during a fourth trial.

With xylazine the horses were more arousable and there was a tendency to more severe postural instability compared to romifidine. Typical cardiopulmonary effects for α_2 -adrenergic agonist were observed with the loading doses, however most of the variables stabilized during the subsequent constant rate infusion. With romifidine some cardiovascular effects were more pronounced and over all effects of romifidine were longer lasting.

In conclusion, quality of sedation was better with romifidine, however in cardiovascularly unstable horses, xylazine might still be advantageous.

2. Introduction

2.1. Standing sedation of horses

Chemical restraint is used daily in equine practice because many diagnostic procedures as well as surgical or medical treatments are impossible without a proper restraint. Horses are not as tolerant to mechanical immobilization as are for example ruminants and they may react violently to excessive restraint, pain or fear. However many procedures can be performed safely on standing horses using combinations of sedative and analgesic drugs, supplemented by appropriate local or regional anaesthesia.

Equine anaesthesia carries a high risk of mortality when compared to general anaesthesia of other species (Johnston et al. 2002; Gibbs & Rodoreda 2005; Bidwell et al. 2007; Brodbelt et al. 2008). Surgical procedures or diagnostic techniques therefore are performed in the standing, sedated horse whenever possible. However, insufficiently sedated horses may not tolerate auditory, tactile, or painful stimuli, and may respond with defensive or aggressive behaviour that can be dangerous for people involved. The goal of sedation for standing surgery is to have a horse that is calm, sedated and indifferent to environmental or noxious stimulation as well as to physical manipulation. The horse should remain standing and only mild ataxia is acceptable. Sedation must be reliable and ideally the horse should not be arousable by noise, touch, handling or movement. On the other hand side effects produced by the sedatives should be minimal.

Due to their characteristics, α_2 -adrenoreceptor agonists are probably the most commonly used and reliable drugs for standing sedation of horses.

2.2. Alpha₂-adrenoreceptor agonists

Horses commonly are sedated with a single bolus of an α_2 -adrenergic agonist or, more usually, a combination of α_2 -adrenergic agonist with an opioid agent. The most frequently used α_2 -adrenoreceptor agonists in equine practice are xylazine, detomidine and romifidine. Medetomidine and dexmedetomidine, even if not registered for horses, have been used successfully in this species (Bettschart-Wolfensberger et al. 1999a, 1999b, 2005; Solano et al. 2009).

2.2.1. Mechanism of action leading to sedation and analgesia

α_2 -adrenoreceptor agonists provide reliable and profound sedation with analgesia and muscle relaxation. The sedative effect of α_2 -adrenoreceptor agonists is due to activation

of α_2 -adrenoreceptors located on locus coeruleus in the pons of the brainstem. The exact mechanism for antinociception is not completely understood, both supraspinal and spinal sites of action seem to be involved (Murrell & Hellebrekers 2005). The increased sensitivity to touch reported clinically after α_2 -adrenoreceptor agonists (England & Clarke 1996) may be due to an effect on the activity of fast conducting non-nociceptive afferent A $_{\beta}$ fibres and seems to be strongest and longest with detomidine compared to romifidine and xylazine (Rohrbach et al. 2009).

2.2.2. Other effects of α_2 -adrenoreceptor agonists

Administration of α_2 -adrenoreceptor agonists is associated with dose dependent cardiovascular side effects including changes in blood pressure and vascular resistances, decreases in cardiac output and decrease in heart rate with development of first- and second-degree atrioventricular block and occasionally other bradyarrhythmias. Changes in blood pressure are characterized by an initial hypertension followed by hypotension (Wagner et al. 1991; Bryant et al. 1996; England & Clarke 1996; Bryant et al. 1998; Bettschart-Wolfensberger et al. 1999b; Yamashita et al. 2000; Freeman et al. 2002; Bettschart-Wolfensberger et al. 2005).

Respiration is slightly depressed, but the effect is usually clinically not relevant unless other drugs are co-administered or anaesthesia is induced. Some horses may demonstrate signs of increased inspiratory effort or begin to snore with duration of sedation as a result of facial and nasal edema due to a lowered head position and relaxation of the muscles in the larynx and nares (Freeman & England 1999; Bryant et al. 1991; Lavoie et al. 1992).

Other side effects of α_2 -adrenoreceptor agonists are reduced gastrointestinal function, hyperglycaemia with decreased serum insulin levels and increased urine output (England & Clarke 1996). The penis is relaxed and extended in males and the uterine tone increased in females (England & Clarke 1996). However the use of α_2 -adrenoreceptor agonists has not been associated to an increased risk of abortion in horses (Katila & Oijala 1988; Luukkanen et al. 1997).

Normally horses do not become recumbent but ataxia may be severe and some cases of falling down have been described after α_2 -adrenoreceptor agonists (Freeman & England 2000)

and after a combination of high doses of α_2 -adrenoreceptor agonists with opioids (Paton & Clarke 1986; Clarke & Paton 1988; Greene & Thurmon 1988).

2.2.3. Combinations with other drugs

Horses sedated with α_2 -adrenergic agonists may respond suddenly to stimulation, especially to touch (England & Clarke 1996). This response, if unexpected, may be dangerous both to the horse and people involved. The combination of opioid drugs with α_2 -adrenergic agonists appears to reduce such sudden reactions and a synergistic effect regarding sedation and antinociception has been suggested (England & Clarke 1996; Schatzman et al. 2001; Kohler et al. 2004; Corletto et al. 2005; Kruluc & Nemec 2006; DeRossi et al. 2009). If high doses of opioids are used, side effects such as excitement (muscle twitching, muzzle tremors, head pressing, increased locomotor activity and circling) can be observed (Muir et al. 1979a; Paton & Clarke 1986; Dyson et al. 1987; Clarke & Paton 1988; Clarke et al. 1991). Also ataxia may be increased after addition of higher doses of opioids to α_2 -adrenoreceptor agonists (Paton & Clarke 1986; Clarke & Paton 1988; Greene & Thurmon 1988). All α_2 -adrenergic agonists have a dose-dependent effect on cardiovascular function (Yamashita et al. 2000). Therefore, by adding an opioid drug, dose requirements of α_2 -adrenergic agonists may be reduced and thus cardiovascular function improved. The addition of opioids does not further impair cardiovascular function (Clarke et al. 1991; Rutkowski et al. 1991). Changes in blood gases have been observed after the addition of opioids to α_2 -adrenoreceptor agonists, but not to a clinically relevant extent (Clarke et al. 1991; Nyman et al. 2009). Based on the reported improved quality of sedation without additional cardiovascular side effects, in clinical practice α_2 -adrenoreceptor agonists are routinely combined with opioids.

Butorphanol tartrate is a synthetic opioid with agonist-antagonist properties. It is convenient to use, as in most countries it is not subject to such stringent controls as are some other opioids. The combination of xylazine and butorphanol at the doses generally used in clinical practice produces minimal and transient haemodynamic effects and no significant respiratory depression (Robertson & Muir 1983). When romifidine is combined with butorphanol experimentally, the quality of sedation and analgesia is better than after romifidine alone (Clarke et al. 1991; Kohler et al. 2004; Holopherne et al. 2005; DeRossi et al. 2009). The combination has also been used successfully in clinical patients (Browning & Collins 1994) and the addition of butorphanol to romifidine did not alter the cardiovascular parameters beyond the changes induced by romifidine alone (Clarke et al. 1991). The successful use of a

butorphanol-detomidine combination has been described in experimental horses (Clarke & Paton 1988) and in clinical patients (Taylor et al. 1988). An improved analgesic effect has been shown by adding butorphanol to detomidine (Schatzmann et al. 2001).

Acepromazine has also been combined with α_2 -adrenoreceptor agonists or added to an α_2 -adrenoreceptor agonist – opioid combination to improve sedation (Muir et al. 1979b; Nilsfors et al. 1988). Unfortunately only few studies are available regarding the effect of acepromazine on horses already sedated with α_2 -adrenoreceptor agonists and no clinical study evaluating the addition of acepromazine exists. The addition of acepromazine to α_2 -adrenoreceptor agonists prevents the initial increase in arterial blood pressure seen when α_2 -adrenoreceptor agonists are used alone (Muir et al. 1979b; Marntell et al. 2005). Also an improved cardiac output and less decrease in heart rate have been reported (Marntell et al. 1996; Marntell et al. 2005). Vasoconstriction induced by α_2 -adrenoreceptor agonists is overcome by acepromazine's vasodilatory properties but the exact mechanism by which acepromazine maintains circulatory variables closer to baseline values during sedation with α_2 -adrenoreceptor agonist remains unclear (Marntell et al. 2005). Also arterial oxygenation is better if acepromazine is added (Marntell et al. 2005).

Opioids, acepromazine and ketamine have been used in combination with CRIs of α_2 -adrenoreceptor agonist for prolonged sedation (Wilson et al. 2002; van Dijk et al. 2003; Solano et al. 2009).

2.2.4. Prolonged sedation: Advantages of a Constant Rate Infusion (CRI)

The use of α_2 -adrenoreceptor agonists as a CRI is becoming more and more popular. At commonly used bolus doses of α_2 -adrenergic agonists, duration of action is usually short, and therefore even for minor interventions, re-dosing is frequently necessary. Therefore, a constant rate infusion (CRI) providing constant plasma concentrations and stable sedation seems advantageous.

Important cardiopulmonary effects occur after a single dose of α_2 -adrenergic agonists, and probably reoccur with each additional dose. Unfortunately there are no prospective, controlled studies about the cardiovascular effects of repeated bolus administration in horses. However, Daunt et al. (1993) described that cardiovascular effects were more severe whenever plasma concentrations of detomidine were increased using a TCI system, and this might mimic

repeated boli administration. On the other hand, Bettschart-Wolfensberger et al. (1999b) described that the cardiovascular effects observed after a loading dose of medetomidine are minimal during subsequent CRI, while sedation is maintained. Therefore, a CRI of α_2 -adrenergic agonists is likely to be safer than the administration of repeated boluses as well as allowing a more constant level of sedation and resultant improved flow of work during surgical procedures.

Constant rate infusion protocols have been described for some of the most commonly used α_2 -adrenoreceptor agonists in horses:

2.2.4.1 Detomidine (see Table 1)

Detomidine has been used as CRI for prolonged standing sedation in experimental horses (Daunt et al. 1993; Aguiar et al. 2009) and clinical cases (Wertz et al. 1994; Wilson et al. 2002). Also the combined use with buprenorphine (van Dijk et al. 2003) or butorphanol (Aguiar et al. 2009) has been reported.

In the proceedings of the BEVA conference 2007 Abrahamsen reported his personal experience by combining detomidine with ketamine and an opioid (morphine or butorphanol). However, when ketamine is used in standing horses, a loading dose should be avoided (personal experience).

Table 1: Detomidine CRIs described for sedation in horses

Drugs	Loading dose ($\mu\text{g kg}^{-1}$; IV)	CRI ($\mu\text{g kg}^{-1}\text{hour}^{-1}$; IV)	If insufficient sedation ($\mu\text{g kg}^{-1}$; IV)	Reference
Detomidine	7.5 ± 1.87	36; dose halved every 15 minutes	6 detomidine or 19 butorphanol	Wilson et al. 2002
Detomidine or Detomidine + Butorphanol	8 8 20	40 20 13		Aguiar et al. 2009
Detomidine + Buprenorphine	10 6	4.2 - 9.6 (mean: 6 ± 0.6)		van Dijk et al. 2003
Detomidine + Ketamine + Morphine or Butorphanol	11 – 22 None 100 – 150 11 – 16	22 600 25 – 50 22	2.2 – 4.4 16.7 – 34 5 – 7	Abrahamsen 2007

2.2.4.3 Medetomidin and Dexmedetomidine (see Table 2)

For medetomidine, despite not being registered for horses, a protocol providing constant sedation and plasma concentrations has been developed in research horses (Bettschart-Wolfensberger et al. 1999a). A medetomidine CRI has also been used in combination with morphine for prolonged sedation during exploratory laparoscopies (Solano et al. 2009).

A CRI of Dexmedetomidine has not been reported for standing sedation of horses.

Table 2: Medetomidine CRIs described for sedation in horses

Drugs	Loading dose ($\mu\text{g kg}^{-1}$; IV)	CRI ($\mu\text{g kg}^{-1}\text{hour}^{-1}$; IV)	If insufficient sedation ($\mu\text{g kg}^{-1}$; IV)	Reference
Medetomidine	5	3.5		Bettschart-Wolfensberger et al., 1999a
Medetomidine + Morphine	5 after 10 minutes: 50	5 30	0.6	Solano et al., 2009

2.2.4.3 Xylazine

Xylazine is the shortest acting α_2 -adrenergic agonist (England et al. 1992). Despite the fact that xylazine, contrary to medetomidine, has been registered for use in horses since 1970, its use as CRI for standing sedation has not been studied extensively. A CRI of xylazine has been used during a xylazine antagonism study (Kollias-Baker et al. 1993), the dose rate used ($0.72 \text{ mg kg}^{-1}\text{h}^{-1}$) being calculated based on experimental data from kinetic studies, but to our knowledge, there are no other published studies.

2.2.4.4 Romifidine

Romifidine is the newest α_2 -adrenergic agonist registered as equine sedative and pre-anaesthetic agent (England et al. 1992; Hamm et al. 1995; Kerr et al. 1996; Freeman et al. 2000). Compared to medetomidine, romifidine has a Marketing Authorisation for administration to horses, and compared to xylazine and detomidine it produces less ataxia at equisedative doses (England et al. 1992; Hamm et al. 1995). Despite these advantages, romifidine has not been described as a CRI for standing sedation of horses.

2.3 Aim of the present thesis

Based on the urgent need of safer sedation protocols for horses and based on the advantages of xylazine and romifidine over other α_2 -adrenoreceptor agonists, four research trials were performed.

The specific aim of the first two trials was the elaboration of CRI dosing regimens for xylazine and romifidine, respectively, to provide deep, constant sedation and steady state plasma concentrations in standing horses. The second goal of the first two studies was to investigate the effects of adding butorphanol to the α_2 -adrenoreceptor agonist CRIs. Our hypothesis was that CRI protocols providing constant sedation and plasma concentrations could be developed for xylazine and romifidine. Further we hypothesized that by adding butorphanol the α_2 -adrenoreceptor agonist requirements would be reduced, and therefore also the dose-dependent cardiovascular effects (Aguair et al. 2009).

During a third trial the elaborated xylazine and romifidine infusions were compared regarding degree of ataxia, depth of sedation and reaction to external stimuli. Our hypotheses for the third trial were equal degree of sedation between the two protocols but less ataxia with romifidine.

Finally, the two α_2 -adrenoreceptor agonist CRIs were compared regarding cardiopulmonary function in a fourth study. Our hypothesis was that the cardiovascular effects due to a loading dose would stabilize during subsequent CRI and that cardiovascular effects would be less with xylazine compared to romifidine.

The goal of the four trials was the development of a safe sedation protocol providing constant sedation that could be prolonged as much as required.

3. Results

3.1. Trial 1: Development of a xylazine constant rate infusion (CRI) with or without butorphanol for standing sedation of horses.

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3.1.1. Summary

Objective: To elaborate CRI protocols for xylazine (X) and xylazine/butorphanol (XB) resulting in constant sedation and xylazine plasma concentrations.

Study design: Blinded randomized experimental study

Animals: Ten adult research horses

Methods: Part I: After normal height of head above ground (HHAG=100%) was determined, a loading dose of xylazine (1 mg kg⁻¹) with butorphanol (XB: 18 µg kg⁻¹) or saline (X: equal volume) was given slowly intravenously (IV). Immediately afterwards, a CRI of butorphanol (XB: 25 µg kg⁻¹hour⁻¹) or saline (X) was administered for 2hours. The HHAG was used as a marker of depth of sedation. Sedation was maintained for 2hours by administration of additional boli of xylazine (0.3 mg kg⁻¹) whenever HHAG>50%. The dose rate of xylazine (mg kg⁻¹hour⁻¹) required to maintain sedation was calculated for both groups. Part II: After the initial loading dose, the calculated xylazine infusion rates were administered in parallel to butorphanol (XB) or saline (X) and sedation evaluated. Xylazine plasma concentrations were measured by HPLC-MS-MS at time points 0, 5, 30, 45, 60, 90, and 120 minutes. Data were analyzed using paired t-test, Wilcoxon signed rank test and a 2-way ANOVA for repeated measures (p<0.05).

Results: There was no significant difference in xylazine requirements (X: 0.69, XB: 0.65 mg kg⁻¹hour⁻¹). With treatment X, a CRI leading to prolonged sedation was developed. With XB, five horses (part I: 2, part II: 3) fell down and during part II four horses were insufficiently sedated. Plasma concentrations were constant after 45 minutes in both groups.

Conclusion: Xylazine bolus, followed by CRI, provided constant sedation. Additional butorphanol was ineffective in reducing xylazine requirements and increased ataxia and early recovery from sedation in unstimulated horses.

Clinical relevance: Data were obtained on unstimulated healthy horses and extrapolation to clinical conditions must be done with caution.

3.1.2. Introduction:

Equine anaesthesia carries a high risk of mortality when compared to general anaesthesia in many species, the overall mortality in horses being 0.24 - 1.9% (Johnston et al. 2002; 2004; Bidwell et al. 2007) compared to 0.17 - 0.24% in cats and dogs (Brodbeck et al. 2008) and < 0.002% in humans (Gibbs & Rodoreda 2005). Surgical procedures or diagnostic techniques therefore are performed in the standing, sedated horse whenever possible because of this high risk. However, insufficiently sedated horses undergoing diagnostic procedures or surgical interventions may not tolerate auditory, tactile, or painful stimuli, and may respond with defensive or aggressive behaviour that can be dangerous for people involved. Thus a reliable sedation protocol is essential.

Horses commonly are sedated with a single bolus dose of an α_2 -adrenergic agonist or, more usually, a combination of α_2 -adrenergic agonist with an opioid agent. However, at the doses used, duration of action is usually short, and repeated boluses of α_2 -adrenergic agonists often are required even for minor surgical procedures. Bolus doses of α_2 -adrenergic agonists cause marked changes in vessel tone, together with bradycardia and a consequential fall in cardiac output (England & Clarke, 1996, Bettschart-Wolfensberger et al. 1999b). However, with a constant rate infusion (CRI) repeated dosing is unnecessary and, at least with the α_2 -adrenergic agonist medetomidine, (Bettschart-Wolfensberger et al. 1999b), cardiopulmonary function is well maintained. Therefore, a CRI of α_2 -adrenergic agonists is likely to be safer than the administration of repeated boluses as well as allowing a more constant level of sedation and resultant improved flow of work during surgical procedures. To date, only the α_2 -adrenergic agonists detomidine and medetomidine have been studied as CRIs for prolonged standing sedation (Daunt et al. 1993; Wertz et al. 1994; Bettschart-Wolfensberger et al. 1999b; Aguiar et al. 2009; Solano et al. 2009). Xylazine is the shortest acting α_2 -adrenergic agonist (England et al. 1992). Despite the fact that xylazine, contrary to medetomidine, has been registered for use in horses since 1970, its use as CRI for standing sedation has not been studied extensively. A CRI of xylazine has been used during a

xylazine antagonism study (Kollias-Baker et al. 1993), the dose rate used being calculated based on experimental data from kinetic studies, but to our knowledge, there are no other published studies.

A problem with horses sedated with α_2 -adrenergic agonists is that they may respond suddenly to stimulation, especially to touch (England et al. 1992). This response, if unexpected, may be dangerous both to the horse and people involved. The combination of opioid drugs with the α_2 -adrenergic agonists appears to reduce such sudden reactions and a synergistic effect regarding sedation and antinociception has been suggested (England et al. 1992; Schatzman et al. 2001; Kohler et al. 2004; Corletto et al. 2005; Kruluc & Nemec 2006; DeRossi et al. 2009). Additionally, all α_2 -adrenergic agonists have a dose-dependent effect on cardiovascular function (Yamashita et al. 2000). By adding an opioid drug, dose requirements of α_2 -adrenergic agonists may be reduced and cardiopulmonary function improved. The addition of opioids does not further impair cardiovascular function (Clarke et al. 1991; Rutkowski et al. 1991). A CRI resulting in constant plasma concentrations in the range associated with analgesia without important side effects has been developed for the opioid butorphanol (Sellon et al. 2001). Butorphanol tartrate is a synthetic opioid with agonist-antagonist properties. It is convenient to use, as in most countries it is not subject to such stringent controls as are some other opioids. Furthermore the combination of xylazine and butorphanol at the doses generally used in clinical practice produces minimal and transient haemodynamic effects and no significant respiratory depression (Robertson & Muir 1983). To our knowledge, there is no ‘blinded’ randomized crossover study regarding the xylazine sparing effects of butorphanol on sedation of horses.

The aim of this study was to elaborate CRI dosing protocols for xylazine and xylazine combined with butorphanol respectively in standing horses, such as to provide deep, constant sedation and steady state plasma concentrations of xylazine. The study was carried out in two parts. In part I, the doses of xylazine necessary to produce a constant level of sedation were found, and a suitable infusion dose calculated from these. In part II, this infusion rate was tested to see if it gave constant sedation, and xylazine plasma concentrations were measured. We hypothesized that a CRI of xylazine can provide constant sedation and constant plasma concentrations and that butorphanol reduces xylazine dose requirements.

3.1.3. Materials and Methods

This study was approved by the Ethical Committee of the National Veterinary School of Lyon (N°0807, May 13th 2008).

The study was carried out in two parts.

Part I

Ten healthy adult research horses (3 geldings, 7 mares) of different breeds (7 French Standardbreds, 2 French Saddlebreds, 1 Thoroughbred) were included in the study. Age was 7.8 ± 1.4 years (mean \pm SD) and a body weight of 522.7 ± 41.2 kg. Horses were considered healthy based on physical examination, complete blood count and serum biochemistry analyses. During the trial, the horses were maintained in groups on pasture with free access to hay and water.

The night before the experiment, the horses were stabled individually to become used to the environment. They were weighed and the region of the neck was clipped for jugular catheter placement.

The following morning, rectal temperature, heart rate and respiratory rate were measured, heart and lungs were auscultated, and mucous membranes and skin turgor evaluated. Subsequently, a 14 gauge x 160 mm catheter (SecalonT; Ohmeda, UK) was inserted percutaneously into one of the jugular veins following aseptic preparation of the skin and subcutaneous infiltration of 1 – 2 mL of lidocaine (Xylovet, CEVA Santé Animale, France). A long extension set consisting of three infusion extension sets (Heidelberger Verlängerung, B. Braun AG, Germany) was connected to the catheter so that drugs could be administered from outside of the box without disturbing the horses. The horses were tied loosely to a ring in the wall throughout the procedure, until complete recovery from sedation. After catheter placement, they were left undisturbed for two hours to get used to the situation. The horses (still undisturbed) were then observed continuously for one hour to assess normal position of nostrils in relation to the ground. The position of the nostrils most frequently observed was marked on the wall. The distance from this mark to the floor was then measured and defined as 100% head height above ground (100% HHAG). The horses were then observed for another 30 minutes to confirm the 100% HHAG. For this purpose, a 10% deviation from the 100% HHAG was marked on the wall. If the horses' nostrils did not stay within 10% of the 100% HHAG, the above procedure was repeated until the $100 \pm 10\%$ HHAG was identified. Once the 100% HHAG was confirmed, the distance from the floor to the 100% HHAG line was divided into 10 equal parts and the respective scale was marked on the wall in front of each horse (Fig. 1).

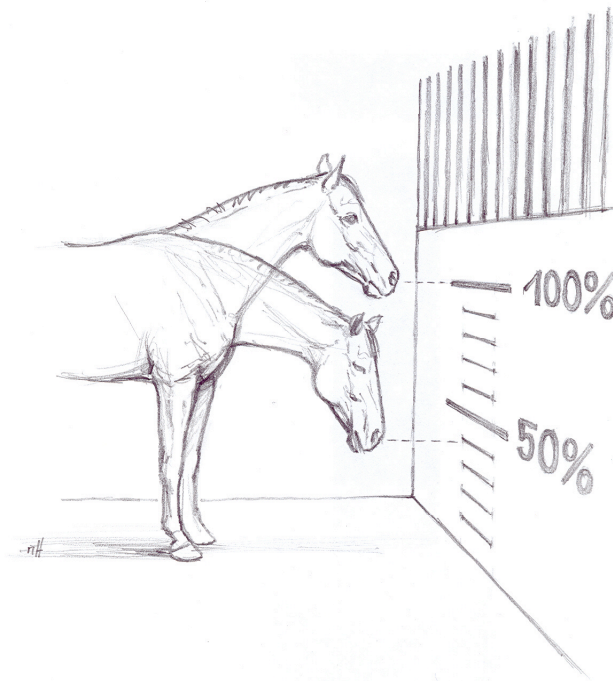


Figure 1: The head height above ground (HHAG) as a marker of depth of sedation. The HHAG is defined as the position of the nose in relation to a scale marked on the wall. The scale is individual to each horse and 100% indicates the normal head position ($\pm 10\%$) of the particular horse while completely awake. A HHAG $\leq 50\%$ indicates sufficient sedation.

The study design was an experimental crossover trial. The horses were assigned randomly to one of two treatment groups (X, XB). Each horse received each treatment with a gap of at least 5 days between treatments. The observer (SKR) was unaware of the treatment given.

All horses were sedated with 1 mg kg^{-1} xylazine hydrochloride (Xylasol ad us. vet., Dr. E. Graeb AG, Switzerland) given intravenously (IV) over 3 minutes by manual injection via the extension set from outside the box stall. The xylazine was followed either by a bolus of butorphanol (Morphasol-10 ad us. vet., Dr. E. Graeb AG, Switzerland) ($18 \text{ } \mu\text{g kg}^{-1}$) diluted in 0.9% saline (Chlorure de Sodium 0.9% B. Braun, B. Braun Medical, France) to a volume of 5 mL (treatment XB) or by an equal volume of saline (treatment X). Thereafter, a CRI of butorphanol ($25 \text{ } \mu\text{g kg}^{-1} \text{ hour}^{-1}$, treatment XB) or of an equivalent volume of saline (treatment X) was commenced. The infusions were delivered through extension sets (Original Perfusor-Leitung PE, B. Braun AG, Germany) by an infusion pump (Syramed μSP6000 , Arcomed AG, Switzerland) situated outside of the box. The extension set was connected to the catheter by a

3-way stopcock (Fresenius Kabi AG, Germany) placed in between the catheter and the infusion extension set used for injection of bolus doses. For the next two hours the horses were observed continuously and HHAG was noted every 5 minutes. Sedation was considered sufficient when head position was equal or lower than 50% of the awake (100% HHAG) position (i.e., sufficient sedation = $\text{HHAG} \leq 50\%$) (Fig. 1). When the nostril position was higher than 50% HHAG, another bolus of 0.3 mg kg^{-1} of xylazine hydrochloride was injected IV via the extension set. The horses were kept sedated for the next two hours. At the end of this time, all drug or saline administration ceased, and the times to regain $> 50\%$ HHAG and to regain normal head position (i.e., 100% HHAG during 2 consecutive observations) were noted. Other effects including the number of times the horses urinated, extreme ataxia and muzzle twitching were noted also.

The dose rate of xylazine ($\text{mg kg}^{-1}\text{hour}^{-1}$) required to maintain sedation was calculated for both groups as the total dose of additional xylazine used after the initial loading dose, divided by the total time the horses were sufficiently sedated ($\text{HHAG} \leq 50\%$).

The two groups were compared for total amount of xylazine needed, time to first additional bolus of xylazine, xylazine requirements during the first hour of sedation, time to normal head position after the last bolus of xylazine, number of urinations, and other effects. The statistical analyses were performed using the software packages SigmaStat[®] 3.5 (SigmaStat[®] 3.5, Systat Software GmbH, Germany) and NCSS (NCSS 2007, UT, USA). Normality was tested by plotting data graphically and by the Kolomogrov-Smirnov normality test. A paired t-test was used to compare the two treatments for normally distributed data (dose rate of xylazine ($\text{mg kg}^{-1}\text{hour}^{-1}$), time until first additional xylazine needed, xylazine requirements during the first hour of sedation, and time to recovery after the last xylazine administration).

Part II

Two of the horses included in part I could not be included in the second part for reasons unrelated to the study. Other research horses substituted these two horses. Horses were weighted and health status assessed as described previously. The group used for part II consisted of four geldings and 6 mares (8 French Standardbreds, 1 French Saddlebred, 1 Thoroughbred), mean body weight $530.8 \pm \text{SD } 44 \text{ kg}$ and age $8.2 \pm 1.7 \text{ years}$.

As for part I, the horses were weighed, clipped for jugular catheter placement, and placed in individual stables the night before the experiment. On the morning of the experiment, the horses were tied loosely to the wall throughout the procedure, until complete recovery from sedation. Two 14 gauge x 160 mm catheters were inserted percutaneously, one into each

jugular vein as described for part I. One of the catheters was designated for drug administration and the other one exclusively for blood sampling. After catheter placement, horses were left undisturbed for two hours. The 100% HHAG was assessed individually for each horse by the same method as described in part I and a scale was marked on the wall.

As for part I, the study design was an experimental crossover. The horses were assigned randomly to one of two treatments (X, XB). Each horse received each treatment with a gap of at least 5 days between treatments. The observer (SKR) was unaware of the treatment given.

All horses were sedated IV with 1 mg kg⁻¹ xylazine hydrochloride given over 3 minutes. The xylazine was followed by a bolus of butorphanol (18 µg kg⁻¹) diluted in 0.9 % saline up to 5 mL (treatment XB) or by an equivalent volume of saline (treatment X). Immediately afterwards, a CRI of butorphanol (25 µg kg⁻¹hour⁻¹, treatment XB) or of an equivalent volume of saline (treatment X) was commenced. Simultaneously, the xylazine infusion rates calculated in part I for treatment X (0.69 mg kg⁻¹hour⁻¹) and XB (0.65 mg kg⁻¹hour⁻¹) were administered, respectively. These CRIs were delivered for 2 hours. All CRIs were delivered using two infusion pumps situated outside the box and through the extension sets connected to the catheter that was designated for drug delivery. Both drug administration extension sets were attached to the same catheter by a 3-way stopcock. Two further extension sets were connected to the sampling catheter so that blood could be withdrawn from outside the box without disturbing the horses.

For the next two hours the horses were observed continuously and HHAG noted every 5 minutes.

In these experiments, ataxia was scored every 5 minutes during the first 30 minutes after starting the bolus administration using a visual analogue scale (VAS). The VAS consisted of a 10 cm line representing no ataxia on the left and the most severe ataxia (falling down) possible on the right.

Blood was sampled for measurement of plasma xylazine concentrations before bolus administration and 5, 30, 45, 60, 90 and 120 minutes after starting the bolus (loading dose) from the catheter that was designated for blood sampling. Thirty millilitres of blood were discarded before sample collection. Afterwards, the blood was injected immediately into heparinized tubes and centrifuged for plasma collection. Directly afterwards, the plasma was frozen and stored at -50 °C until xylazine concentrations were measured (see Appendix A: Analysis of xylazine hydrochloride plasma concentrations).

Infusions continued for two hours. Once the infusions ceased, the time to regain > 50% HHAG and time to regain normal head position (i.e., 100% HHAG during 2 consecutive observations) were noted.

During the time of the infusions, the times that any horses were not sufficiently sedated (i.e. HHAG > 50%) were noted. The overall sedation was considered insufficient if the head position was higher than 50% at more than 3 out of 25 measurement points or if the last 3 measurement points (110, 115, 120) were > 50%.

Groups were compared by a Wilcoxon signed rank test for nonparametric data (recovery time to > 50% HHAG, recovery time to 100% HHAG). Following testing for normality, ataxia VAS were analysed using a 2-way ANOVA for repeated measures (two within factors) followed by a Wilcoxon signed rank test whenever significance was detected. To determine from which time point onward plasma concentrations were constant and to compare values between treatments, a 2-way ANOVA for repeated measures (two within factors) was used followed by a Tukey test for pairwise multiple comparison. Plasma concentrations were considered constant when no further changes were detected on subsequent measurements. A general linear model was used to study the association between depth of sedation (HHAG) and xylazine plasma concentrations, treatment, and the individual horse. The level of significance was set at $p < 0.05$.

3.1.4. Results

Part I

Extreme ataxia was noted in 2 horses with treatment X and in 5 horses with treatment XB. Two horses fell 20 minutes after starting the loading dose with XB. The first horse stood up immediately. The second horse stayed on the floor for 30 minutes and was therefore excluded from the study.

There were no significant differences in xylazine requirements between the two groups (X: 0.69 ± 0.17 ; XB: 0.65 ± 0.16 mg kg⁻¹hour⁻¹; $p = 0.63$). The time until the first additional bolus of xylazine was administered was not significantly different between the two treatments (X: 35.6 ± 12.6 ; XB: 36.1 ± 12.7 minutes; $p = 0.94$). There were no significant differences in total xylazine requirements during the first hour (X: 1.47 ± 0.22 ; XB: 1.43 ± 0.22 mg kg⁻¹; $p = 0.76$). Time to complete recovery (100% HHAG) after the last bolus of xylazine was not significantly different between the two groups (30.5 ± 9.8 ; XB: 33.33 ± 6.1 minutes; $p = 0.47$).

Shaking and trembling of the head or muzzle was noted in two horses while receiving X and in four horses during XB. One horse receiving treatment X and 3 horses receiving treatment XB were judged to be mildly excited during recovery.

The horses overall urinated 11 times during X and 10 times during XB. The individual horses urinated maximally twice during the study period.

With each treatment, 5 horses had moderate to heavy sweating at the end of sedation.

Part II

There was a significant association between the main effects xylazine plasma concentration ($p < 0.001$), treatment ($p = 0.026$), and horse ($p = 0.037$) with HHAG, but no significant interaction between horse and treatment ($p = 0.28$).

Plasma concentrations (Table 3) were constant 45 minutes after starting the loading dose and there was no significant difference between groups ($p = 0.361$).

Table 3: Mean \pm SD plasma concentrations of xylazine hydrochloride (ng L^{-1}) in 10 research horses before drug administration (0 min), after a loading dose of xylazine 1 mg kg^{-1} (treatment X) or xylazine 1 mg kg^{-1} combined with butorphanol $18 \text{ } \mu\text{g kg}^{-1}$ (treatment XB) IV (5 min), and during a constant rate infusion (3 – 120 min) of xylazine $0.69 \text{ mg kg}^{-1} \text{ hour}^{-1}$ (treatment X); or xylazine $0.65 \text{ mg kg}^{-1} \text{ hour}^{-1}$ combined with butorphanol $25 \text{ } \mu\text{g kg}^{-1} \text{ hour}^{-1}$ (treatment XB).

	0 min	5 min	30 min	45 min	60 min	90 min	120 min
X	0 ± 0	874 ± 162.4	571 ± 93	489 ± 53	466 ± 51	452 ± 34	455 ± 42
XB	0 ± 0	941 ± 177	578 ± 105	513 ± 71	468 ± 58	460 ± 48	465 ± 59

With treatment XB, xylazine plasma concentration of one horse at time point 60 minutes was excluded from further analyses because it was considered an outlier (the analysis had been repeated three times, always obtaining consistently high values that were 5 times the average plasma concentration).

Significant differences regarding degree of ataxia over time and between groups were detected. The horses were significantly more ataxic with treatment XB at time points 15 ($p < 0.001$) and 20 ($p = 0.002$) minutes (Fig. 2).

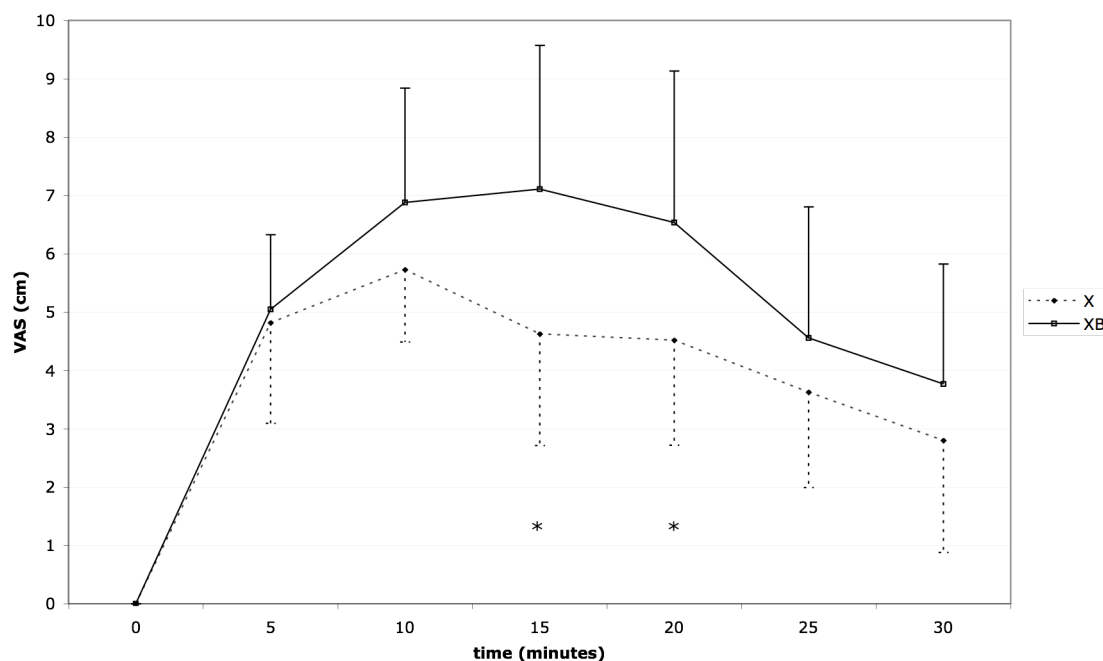
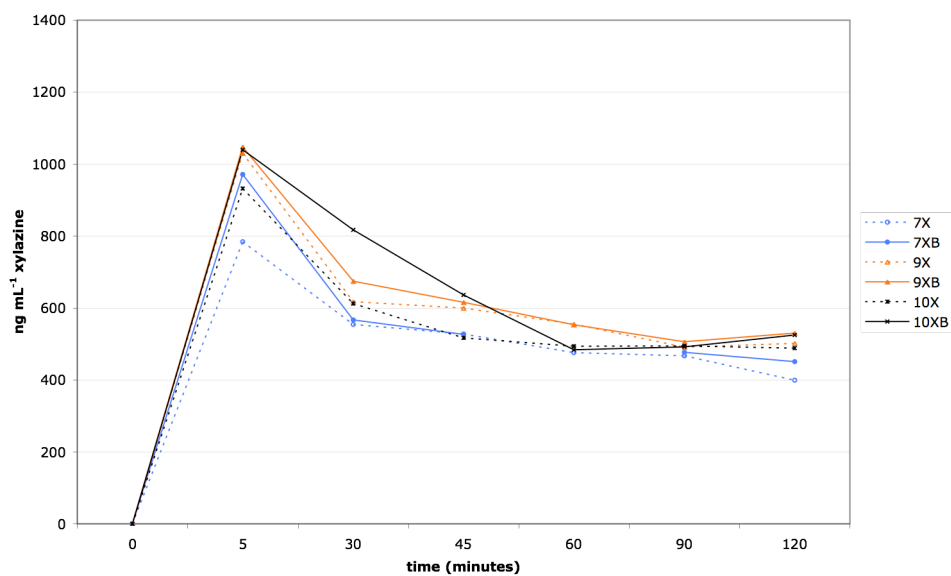


Figure 2: Ataxia assessed by a visual analogue scale (VAS) (0 cm representing no ataxia and 10 cm maximal possible ataxia with the horse falling down) after loading dose of xylazine 1 mg kg⁻¹ (treatment X) or xylazine 1 mg kg⁻¹ combined with 18 µg kg⁻¹ butorphanol (treatment XB) IV followed by a constant rate infusion of xylazine 0.69 mg kg⁻¹ hour⁻¹ (treatment X); or xylazine 0.65 mg kg⁻¹ hour⁻¹ combined with butorphanol 25 µg kg⁻¹ hour⁻¹ (treatment XB) in 10 research horses. Mean ± SD presented.

* statistically significant ($p < 0.05$) difference between treatments.

Three horses receiving treatment XB fell down between 10 – 15 minutes after starting the loading dose. All of the horses stood up immediately and were therefore not excluded from further analysis. One of the horses adopted saw-horse stance after standing up and was therefore excluded from evaluation of HAAG as the nose position was constantly $> 50\%$ despite deep sedation. Two of the three horses that fell down were included in part I and one of these two horses also fell down during part I. The second horse that fell down during part I was not included in part II. Overall, there was no significant difference in plasma xylazine concentrations between XB and X. However, at time points 5 and 30 minutes, the horses that fell down showed higher plasma xylazine concentrations with XB compared to treatment X (Fig. 3a). However the plasma concentrations of these three horses in relation to the whole group were not the highest (Fig. 4).

a)



b)

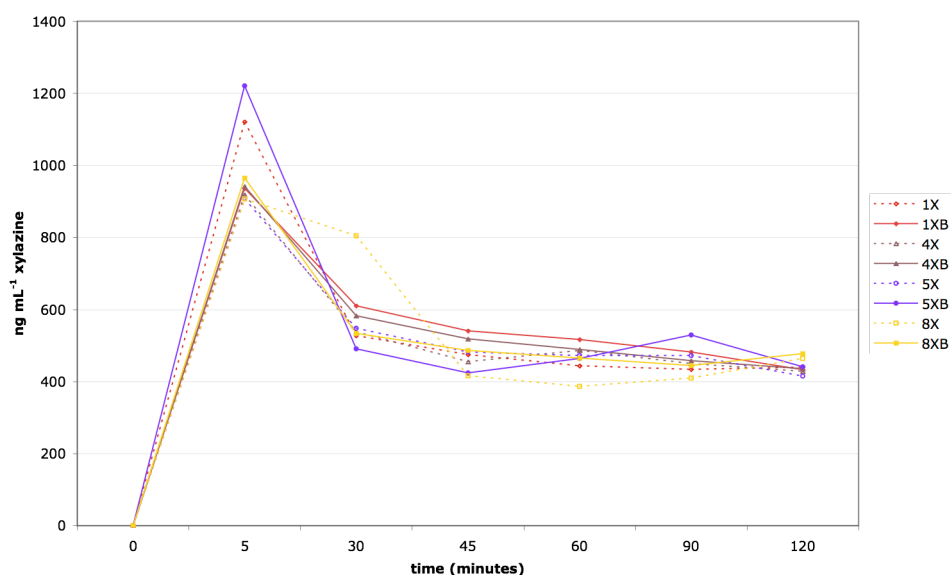


Figure 3: Plasma concentrations of xylazine hydrochloride (ng mL⁻¹) in three horses (horses number 7, 9 and 10) after a loading dose of xylazine 1 mg kg⁻¹ (treatment X); or xylazine 1 mg kg⁻¹ combined with 18 µg kg⁻¹ butorphanol (treatment XB) IV followed by two hours constant rate infusion (CRI) of xylazine 0.69 mg kg⁻¹ hour⁻¹ (treatment X); or xylazine 0.65 mg kg⁻¹ hour⁻¹ combined with butorphanol 25 µg kg⁻¹ hour⁻¹ (treatment XB). With XB (dark lines) the horses fell down 20 minutes after starting the loading dose.

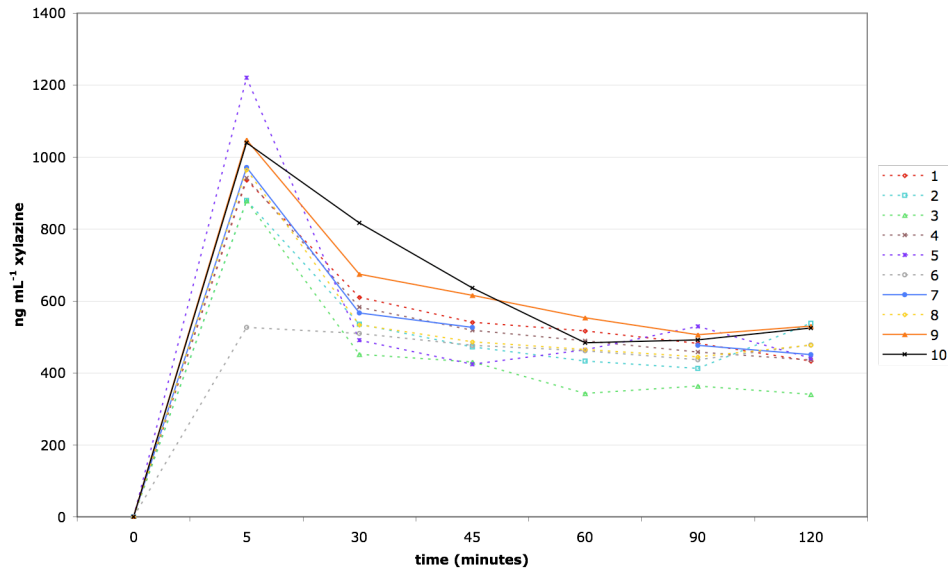


Figure 4: Plasma concentrations of xylazine hydrochloride (ng mL^{-1}) in ten horses after a loading dose of 1 mg kg^{-1} xylazine combined with $18 \text{ } \mu\text{g kg}^{-1}$ butorphanol (treatment XB) IV followed by two hours constant rate infusion (CRI) of xylazine $0.65 \text{ mg kg}^{-1} \text{ hour}^{-1}$ and butorphanol $25 \text{ } \mu\text{g kg}^{-1} \text{ hour}^{-1}$ (treatment XB). Horses 7, 9 and 10 (dark, continuous lines) fell down 20 minutes after starting the loading dose.

Four horses, all of them with treatment XB, were considered insufficiently sedated. Three of the horses presented HHAG $> 50\%$ consistently until the end of the sedation period starting 70, 105 and 95 minutes after starting the administration of the loading dose. One horse was considered insufficiently sedated between time points 65 to 105 minutes, afterwards it became re-sedated (HHAG $\leq 50\%$) from 110 – 125 minutes. The horses presenting insufficient sedation with XB, did not have overall lower plasma concentrations when compared to treatment X where they were sufficiently sedated (Fig. 3b). The awakening with XB did not coincide with lower xylazine plasma concentrations compared to treatment X. No horse showing insufficient sedation presented plasma concentrations below 400 ng mL^{-1} . With XB, one horse had plasma concentrations below 400 ng mL^{-1} from 60 minutes onward but was sufficiently sedated during that time period. With treatment X, 3 different horses presented plasma concentrations below 400 ng mL^{-1} , only one of them remaining consistently below 400 ng mL^{-1} until the last measurement point (120 minutes). This particular horse was sufficiently sedated until the end of the 120 minutes and remained sufficiently sedated until 20 minutes after discontinuing CRI. All four horses presenting insufficient sedation with XB in the second part of the study had been included in the development of the dosing regime during part I.

In total, the horses urinated 18 times during X and 23 times during XB. The maximal number of times any one horse urinated was four.

3.1.5. Discussion

In the present study a CRI regime of xylazine that induces a constant level of sedation, in horses, as assessed by the position of the head was elaborated. The combination with butorphanol did not reduce xylazine requirements. However, by adding butorphanol during the first 20 minutes some horses fell and during the second hour of sedation other horses appeared insufficiently sedated. Constant xylazine plasma concentrations were achieved in both groups and there was no significant difference in plasma concentrations between treatments.

Contrary to our results, a synergistic effect regarding sedation (Corletto et al. 2005), analgesia (Robertson & Muir 1983; Schatzman et al. 2001; Kohler et al. 2004) and response to stimulation (Paton & Clarke 1986; Clarke & Paton 1988; Clarke et al. 1991; Holopherne et al. 2005) has been described by adding opioids to α_2 -adrenergic agonists. Based on these results, in clinical situations α_2 -adrenergic agonists are often combined with opioid drugs in order to improve effectiveness of sedation. The methods used in the present study have been used the first time to test the effects of additional butorphanol to α_2 -adrenergic agonists. However, the study design may not be ideal for this purpose. The horses were not stimulated and were pain free, therefore the positive effect that butorphanol may have in clinical cases, could have been missed. Depth, as assessed by head height, and not quality of sedation was tested in the present study.

The dose of butorphanol was selected based on the publications of Sellon et al. which showed that with this dose constant plasma concentrations are achieved inducing analgesia following colic surgery without other untoward effects (Sellon et al. 2001, 2004). There are no other studies investigating constant rate butorphanol infusions in combination with xylazine. Combined with 1.1 mg kg^{-1} xylazine, a single dose of 0.1 mg kg^{-1} butorphanol was effective in improving analgesia during experimental standing surgery (Robertson & Muir 1983). However, 0.04 mg kg^{-1} butorphanol did not improve analgesia in a dental dolometry model (Brunson & Majors 1987). Doses of $0.02 - 0.05 \text{ mg kg}^{-1}$ of butorphanol have been effective in improving analgesia and sedation quality when combined with different α_2 -adrenergic agonists (Paton & Clarke 1986; Clarke & Paton 1988; Clarke et al. 1991; Schatzman et al.

2001; Kohler et al. 2004; Corletto et al. 2005; Holopherne et al. 2005; DeRossi et al. 2009). The effect of a higher dose rate of butorphanol in the current model remains to be tested.

The methodology used in the present study to develop a CRI regime of α_2 -adrenergic agonists to provide constant sedation and plasma concentrations has been proven to be effective with the α_2 -adrenergic agonist medetomidine in horses (Bettschart-Wolfensberger et al. 1999a). Kollias-Baker et al. (1993) used a xylazine CRI of $12 \mu\text{g kg}^{-1} \text{hour}^{-1}$ ($0.72 \text{ mg kg}^{-1} \text{hour}^{-1}$) to achieve plasma concentrations between $400 - 800 \text{ ng mL}^{-1}$ and moderate sedation (Kollias-Baker et al. 1993). The dose rate was calculated based on experimental kinetic data and the resulting dose rate is similar to dose rates calculated during part I of the present study. These authors reported a profound and sustained sedation, which was assessed by a chin to floor distance. Similarly, in our study depth of sedation was assessed by head position above the ground and horses treated with xylazine only (X) were constantly and sufficiently sedated until the end of CRI. Constant plasma concentrations were achieved with both treatments (X, XB) and were between $340 - 636 \text{ ng mL}^{-1}$ during the steady state phase (45 – 120 minutes after starting the loading dose). There was a significant association between HHAG and plasma concentrations of xylazine. However, one horse with XB and three horses with X showed plasma concentrations below 400 ng mL^{-1} but remained sufficiently sedated, probably because HHAG is also significantly associated with the individual horse and treatment.

The insufficient sedation noted with XB may be due to a stimulatory effect of butorphanol. Adverse behavioural effects including mild excitement, restlessness, tossing and jerking of the head, increase locomotor activity, augmented avoidance response to auditory stimuli and ataxia have been observed after administration of high doses of butorphanol to non-painful horses (Robertson et al. 1981; Kalpravidh et al. 1984; Sellon et al. 2001), although, this was observed only if doses exceeded $0.05 \text{ mg kg}^{-1} \text{ IV}$. At doses used in the present study no adverse behavioural effects were observed in healthy horses (Sellon et al. 2001), although no concomitant α_2 -adrenergic agonist was administered. Clarke & Paton (1988) noted signs of excitement in horses given a single dose of detomidine ($10 \mu\text{g kg}^{-1}$) followed by butorphanol (0.05 mg kg^{-1}). Xylazine may have an effect on metabolism and clearance of butorphanol. The cardiovascular effects of xylazine (Wagner et al. 1991; Bueno et al. 1999; Yamashita et al. 2000) may influence blood flow to organs involved in metabolism and

elimination of butorphanol, leading to higher plasma concentrations. Unfortunately plasma concentrations of butorphanol were not measured.

The initial bolus dose of xylazine used in the present study is recommended commonly for sedation and has been used in combination with equal or higher doses of butorphanol in experimental studies (Robertson & Muir 1983; Brunson & Majors 1987; Rutkowski et al. 1991). It was surprising therefore that four different horses fell down on five occasions in both parts of the study, all of them receiving treatment XB. Unacceptable degree of ataxia and/or falling down after α_2 -adrenergic agonist treatment (Freeman & England 2000) or after combination of high doses of α_2 -adrenergic agonist with opioids (Paton & Clarke 1986; Clarke & Paton 1988; Greene & Thurmon 1988) has been observed previously. Paton & Clarke (1986) observed that severe ataxia may be a serious side effect if an opiate was added to horses already ataxic because of an α_2 -adrenergic agonist. These authors defined it as a reduction in the ability to retain their balance and they discussed that the horses seemed to be unaware of this instability if an opiate was added. However, with just the α_2 -adrenergic agonists they seemed to awake and regain balance if they staggered (Paton & Clarke 1986). If this observation is due to a specific effect of the opioid itself or due to a more profound sedation is unknown. Butorphanol on its own can cause ataxia, but only after much higher doses than the ones used in the present study (Robertson et al. 1981; Nolan et al. 1994). A cardiovascular collapse because of the addition of butorphanol seems to be unlikely as butorphanol does not further depress cardiovascular function in horses already sedated with α_2 -adrenergic agonists (Clarke et al. 1991; Rutkowski et al. 1991) and we administered the initial bolus dose slowly over 3 minutes. Compared to the present study, in clinical practice the loading dosage of xylazine is normally slightly reduced when butorphanol is added. However, in order to compare the two treatments we had to start with the same loading dose of xylazine.

The main limitation of the study regarding extrapolation to clinical practice is that we used healthy pain-free experimental horses and did not subject them to any form of stimulation. Differences regarding the effect of butorphanol on xylazine requirements, and probably also the insufficient sedation, may be different in clinical cases where horses may be painful and stimulated.

In conclusion, a loading dose of xylazine (1 mg kg^{-1}) followed by two hours of CRI ($0.69 \text{ mg kg}^{-1} \text{ hour}^{-1}$) provided constant sedation (as judged by head height) and plasma concentrations in healthy unstimulated horses. The dose of butorphanol used in this study was ineffective in decreasing xylazine requirements and may increase the risk of exaggerated ataxia and apparently insufficient sedation in unstimulated horses. Based on our results, the developed XB protocol cannot be recommended. However, results should be extrapolated to clinical conditions with caution as data were obtained from healthy unstimulated horses. Possibly a lower loading dose of xylazine followed by a higher CRI would have provided a better sedation protocol in combination with butorphanol.

Appendix A: Analysis of xylazine hydrochloride plasma concentrations

Reagents and Chemicals - Xylazine was purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile, HPLC grade, was obtained from Merck (Darmstadt, Germany), ammonium formate (mass spectroscopy grade) from Fluka (Steinheim, Germany).

Standard Preparation - An initial standard of xylazine was prepared by weighing 10 mg of the standard and then dissolving it in acetonitrile to get the exact volume of 100 mL. That solution was kept in a freezer at -18°C for no more than a month. It was used, after letting it reach room temperature for about one hour, to prepare diluted standard as needed. $100 \text{ } \mu\text{g mL}^{-1}$, $10 \text{ } \mu\text{g mL}^{-1}$, $1 \text{ } \mu\text{g mL}^{-1}$ and $0.1 \text{ } \mu\text{g mL}^{-1}$ working standard solutions were prepared by dilution in acetonitrile and were used as spiking solutions. A $1 \text{ } \mu\text{g mL}^{-1}$ solution in a mixture of acetonitrile and ammonium formate 10 mM (50:50) was prepared for infusion in the ESI source to optimize the MS conditions.

Samples preparation - Extraction was adapted from the published method of Miksa et al. (Miksa et al. 2005). 250 μL of plasma with the same volume of acetonitrile were prepared in an eppendorf. The mixture was vortexed during 5 seconds, and then left standing for 2 minutes at ambient temperature. Afterwards it was centrifuged at 13 rpm for 5 minutes. The upper layer was transferred to another eppendorf, and centrifuged again with the same conditions. Finally the upper layer was transferred to a vial for LC/MS/MS injection.

LC-ESI/MS/MS - The LC-ESI/MS/MS used was a 1100 Series LC/MSD ion Trap VL with an ElectroSpray Ionisation (ESI) interface and a LCMS Chemstation software from Agilent Technologies (Palo Alto, CA, USA). Chromatographic separation was performed using a Zorbax Eclipse-C18 ($2.1 \text{ mm} \times 10 \text{ mm}$, $3.5 \text{ } \mu\text{m}$) column and a Zorbax Rx-SIL ($2.1 \text{ mm} \times 12.5 \text{ mm}$, $5 \text{ } \mu\text{m}$) guard-column from Agilent Technologies with a mobile phase of A:10 mM ammonium formate, 0.1% formic acid and B:acetonitrile HPLC grade. The mobile phase gradient elution was 70%A:30%B (v:v) at 0 minutes, increasing to 50%A:50%B from 0 to 5 minutes, holding at 80%B from 6 to 7 minutes and back at 30% B at 8 minutes for a total run time of 18 minutes including column equilibration. The column temperature was 30°C . The flow rate in the LC column was $0.25 \text{ mL minute}^{-1}$. The injection volume was $1 \text{ } \mu\text{L}$. The temperature of the auto sampler tray was set to 5°C .

Detection was by MS/MS with electrospray ionization in positive mode. Nebulizer pressure was set to 40 psi, dry gas temperature to 350°C , and dry gas flow to 8 L minute^{-1} . On the basis of infusion experiments, optimized MS instrument conditions were determined. Capillary voltage was set to 3500V, CID to 1V. Collision gas in the trap was helium with a pressure of $0.6 \times 10^{-5} \text{ mbar}$.

Validation of the LC-MS/MS method

Identification - Identification criteria for xylazine were the retention time ($t_r = 2.2$ minutes) and the ion ($m/z = 221$).

Specificity - Specificity was tested with blank horse plasma (10 probes) and there were no interferences to be found.

Limit of quantification - Considering the mean signal to noise ratio $S/B_{(blank)}$ and the standard deviation $\sigma_{(blank)}$ for 10 blank injections, the limit of quantification (LOQ) was determined by the mean of 5 blank samples spiked in such a way we obtained a signal intensity at least equal to $S/B_{(blank)} + 10\sigma_{(blank)}$. The LOQ was 10 ng mL^{-1} .

Linearity - Linearity was tested from 10 to 1000 ng mL^{-1} . Four replicates of spiked blank horse plasma spiked at six different concentrations were performed for a total of 24 injections. The response was linear throughout the concentration range tested with the coefficient of correlation (r^2) equal to 0.9999 , and the calibration curve obtained was $A_{\text{xylazine}} = 1970.01 \cdot C_{\text{xylazine}} + 6368.20$. The peak areas (A_{xylazine}) were then used in conjunction with the calibration curve to determine the concentration of xylazine (C_{xylazine}) in the samples. The molecule present in the injectable form of the drug (Xylasol) is xylazine hydrochloride (mol wt 256.79). We observed that horse plasma with added xylazine hydrochloride (Xylasol) (mol wt 256.79) or xylazine (Sigma) (mol wt 220.33) to get equal xylazine concentrations gave similar peak areas. A correction factor of $256.79/220.33$ was then applied to get final results with xylazine hydrochloride concentrations in ng mL^{-1} .

Precision - The precision was evaluated by performing four replicates of six spiked quality control (QC) samples. The mean intra-day accuracy ($n=4$) for each concentration tested was between 97 and 102% with a coefficient of variation between 2 and 12% , except for the LOQ (10 ng mL^{-1}) where the accuracy was between 75% and the coefficient of variation 11% .

Matrix effects - While infusing 100 ng mL^{-1} xylazine solution, a blank horse plasma was injected and no matrix effects were observed.

3.2 Trial 2: Development of a romifidine constant rate infusion with or without butorphanol for standing sedation of horses.

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3.2.1. Summary

Objective: To determine constant rate infusion (CRI) protocols for romifidine (R) and romifidine combined with butorphanol (RB) resulting in constant sedation and romifidine plasma concentrations.

Study design: Blinded randomized crossover study

Animals: Ten adult research horses

Methods: Part I: After determining normal height of head above ground (HHAG=100%), loading doses of romifidine ($80 \mu\text{g kg}^{-1}$) with butorphanol (RB: $18 \mu\text{g kg}^{-1}$) or saline (R) were given intravenously (IV). Immediately afterwards, a butorphanol (RB: $25 \mu\text{g kg}^{-1}\text{hour}^{-1}$) or saline (R) CRI was administered for 2 hours. The HHAG was used as marker of sedation depth. Sedation was maintained for 2 hours by additional romifidine ($20 \mu\text{g kg}^{-1}$) whenever HHAG>50%. The dose rate of romifidine ($\mu\text{g kg}^{-1}\text{hour}^{-1}$) required to maintain sedation was calculated for both treatments. Part II: After loading doses, the romifidine CRIs derived from part I were administered in parallel to butorphanol (RB) or saline (R). Sedation and ataxia were evaluated periodically. Romifidine plasma concentrations were measured by HPLC-MS-MS at 0, 5, 10, 15, 30, 45, 60, 90, 105, and 120 minutes. Data were analyzed using paired t-test, Fisher's exact test, Wilcoxon signed rank test, and 2-way ANOVA for repeated measures ($p < 0.05$).

Results: There was no significant difference in romifidine requirements (R: 30; RB: $29 \mu\text{g kg}^{-1}\text{hour}^{-1}$). CRI protocols leading to constant sedation were developed. Time to first additional romifidine bolus was significantly longer in RB (R: 38.5 ± 13.6 ; RB: 50.5 ± 11.7 minutes). Constant plasma concentrations of Romifidine were achieved during the second hour of CRI. Ataxia was greater when butorphanol was added.

Conclusion: Romifidine bolus, followed by CRI, provided constant sedation assessed by HHAG. Butorphanol was ineffective in reducing romifidine requirements in unstimulated horses, but prolonged the sedation caused by the initial romifidine bolus.

Clinical relevance: Both protocols need to be tested under clinical conditions.

3.2.2. Introduction

Due to the risks and expenses associated with equine anaesthesia, minor surgical procedures and procedures benefiting from standing position such as laparoscopy are often performed in the standing, sedated horse. For prolonged standing sedation the use of constant rate infusion (CRI) protocols including α_2 -adrenergic agonists are becoming increasingly popular. The α_2 -adrenergic agonists detomidine and medetomidine have been studied as CRIs for sedation of horses (Daunt et al. 1993; Wertz et al. 1994; Bettschart-Wolfensberger et al. 1999a, 1999b; Wilson et al. 2002; van Dijk et al. 2003; Aguiar et al. 2009; Solano et al. 2009). A xylazine CRI protocol has been developed recently in experimental horses (Ringer et al. 2012a).

Romifidine is the α_2 -adrenergic agonist that has most recently been developed as an equine sedative and pre-anaesthetic agent (England et al. 1992; Hamm et al. 1995; Kerr et al. 1996; Freeman et al. 2000). Compared to medetomidine, romifidine has a Marketing Authorisation for administration to horses, and compared to xylazine and detomidine it produces less ataxia at equi-sedative doses (England et al. 1992; Hamm et al. 1995). Despite these advantages romifidine has not been described as a CRI for standing sedation of horses.

When romifidine is combined with butorphanol experimentally, the quality of sedation and analgesia is better than after romifidine alone (Clarke et al. 1991; Kohler et al. 2004; Holopherne et al. 2005; DeRossi et al. 2009). The combination has also been used successfully in clinical patients (Browning & Collins 1994) and the addition of butorphanol to romifidine did not alter the cardiovascular parameters beyond the changes induced by romifidine alone (Clarke et al. 1991).

The aim of this study was to determine CRI dosing protocols for romifidine and romifidine combined with butorphanol, respectively, to provide profound, constant sedation and steady state plasma concentrations of romifidine in standing horses. This current study uses the same methods previously used to develop a xylazine CRI, and to study the effects of butorphanol on xylazine requirements (Ringer et al. 2012a). We hypothesized that a CRI of romifidine

would provide constant sedation and constant plasma concentrations and that butorphanol would reduce romifidine dose requirements.

3.2.3. Materials and Methods

This study was approved by the Ethical Committee of the National Veterinary School of Lyon (N°0807, May 13th 2008). The study was divided in two parts.

Part I

Ten healthy adult research horses (4 geldings, 6 mares) of different breeds (9 French Standardbreds, 1 French Saddlebred) with an age of 7.8 ± 1.6 years (mean \pm SD) and a body mass of 526 ± 44 kg were included in the study.

Horses were kept and prepared as described by Ringer et al. (2012a). The same materials and methods were used for jugular catheter placement and determination of the 100% head height above ground (100% HHAG).

The study was designed as an experimental crossover trial. The horses were randomly assigned to one of two treatment groups (romifidine (R) or romifidine combined with butorphanol (RB)) and one week was left between treatments. The observer (SKR) was unaware of treatment allocation.

Horses were sedated from outside the box with $80 \mu\text{g kg}^{-1}$ romifidine (Sedivet®, Boehringer Ingelheim France, Paris, France) given intravenously (IV) over 3 minutes. The romifidine was followed by a bolus of butorphanol (Morphasol-10 ad us. vet., Dr. E. Graeub AG, Bern, Switzerland) ($18 \mu\text{g kg}^{-1}$) diluted in 0.9% saline (Chlorure de Sodium 0.9% B. Braun, B. Braun Medical, Boulogne Cedex, France) to a volume of 5 mL (treatment RB) or by an equal amount of saline (treatment R). Thereafter, a CRI of butorphanol ($25 \mu\text{g kg}^{-1}\text{hour}^{-1}$, treatment RB) or an equivalent volume of saline (treatment R) was started. For the next two hours the horses were continuously observed and the HHAG was noted every 5 minutes. Sedation was considered sufficient when head position was equal or lower than 50% of the awake (100% HHAG) position (i.e., sufficient sedation = $\text{HHAG} \leq 50\%$). When the nostril position was higher than 50% HHAG, another bolus of $20 \mu\text{g kg}^{-1}$ of romifidine was injected IV. In this way the horses were kept sedated for the next two hours.

At the end, the total time the horses were sufficiently sedated (i.e., $50\% \leq \text{HHAG}$) was noted. The total time of sufficient sedation was defined as starting with the loading dose administration and ending with the first observation of a $\text{HHAG} > 50\%$ after the two hours of sedation. The number of times the horses urinated, as well as observations like extreme ataxia and muzzle twitching were noted also.

The dose rate of romifidine ($\mu\text{g kg}^{-1}\text{hour}^{-1}$) required to maintain sedation was calculated for both groups as the total dose of additional romifidine used after the initial loading dose, divided by the total time the horses were sufficiently sedated ($\text{HHAG} \leq 50\%$).

Ataxia was scored using a visual analogue scale (VAS) every 5 minutes during the first 30 minutes after the initial dose of romifidine. The VAS consisted of a 10 cm line representing no ataxia on the left (0 cm) and the most severe ataxia possible (falling over) on the right (10 cm). The observer placed a mark on the line that corresponded to the ataxia of the individual horses at the different measurement points.

Statistical analyses were performed using the software packages SigmaStat® 3.5 (SigmaStat® 3.5, Systat Software GmbH, Ekrath, Germany) and NCSS (NCSS 2007, Kaysville, USA). Normality was tested by plotting data graphically and by using the Kolomogrov-Smirnov normality test. A paired t-test was used to compare the two treatments for normally distributed data (dose rate of romifidine ($\mu\text{g kg}^{-1}\text{hour}^{-1}$), time until first additional romifidine needed and total amount of romifidine used during the first hour). Ataxia VAS were analysed using a 2-way ANOVA for repeated measures (two within factors) followed by a Wilcoxon signed rank test whenever significance was detected. The number of horses observed with shaking/trembling of the head or muzzle was compared between the two groups using a Fisher's exact test. The level of significance was set at $p < 0.05$.

Part II

Horses were weighted again (526 ± 54.1 kg) and their health status was assessed.

The horses were prepared in the same way as described by Ringer et al. (2012a). Two jugular catheters were inserted, one into each jugular vein. One of the catheters was designated for drug administration and the other one exclusively for blood sampling. Two hours after catheter placement the 100% HHAG was assessed and a scale (0 – 100% HHAG) marked on the wall.

The study was designed as an experimental crossover trial. The horses were randomly assigned to one of the two treatment groups (R, RB) with a gap of one week between treatments. The observer (SKR) was unaware of treatment allocation.

The same loading doses as used during part I were administered IV. Immediately afterwards, a CRI of butorphanol ($25 \mu\text{g kg}^{-1}\text{hour}^{-1}$, treatment RB) or an equivalent volume of saline (treatment R) was started. Simultaneously, the romifidine infusion rates calculated in part I for treatment R ($30 \mu\text{g kg}^{-1}\text{hour}^{-1}$) and RB ($29 \mu\text{g kg}^{-1}\text{hour}^{-1}$) were administered, respectively. Constant rate infusions were delivered for 2 hours. The infusions (romifidine and butorphanol

or saline) were delivered by two infusion pumps situated outside the box and through the extension sets connected to the catheter that was designated for drug delivery. Two infusion extension sets were connected to the sampling catheter so that blood could be withdrawn from outside the box without disturbing the horses.

For the next two hours the horses were observed continuously and the HHAG noted every 5 minutes.

Ataxia while standing was scored by VAS every 5 minutes during the first 30 minutes after the bolus administration.

Blood was sampled for measurement of plasma romifidine concentrations before bolus administration and 5, 10, 15, 30, 45, 60, 90, 105 and 120 minutes after starting the bolus (loading dose) from the catheter that was designated for blood sampling. Thirty millilitres of blood was discarded before sample collection. The blood sample was injected immediately into heparinized tubes and centrifuged (2000 g for 10 min) (TJ-6 Centrifuge, Beckman Coulter France S.A.S., Villepinte, France) to enable plasma collection. Directly afterwards, the plasma was frozen and stored at -50°C until romifidine hydrochloride concentrations were measured (see Appendix B: Analysis of romifidine plasma concentrations).

At the end, the time (after discontinuing CRI) to regain $> 50\%$ HHAG and to regain normal head position (i.e. 100% HHAG) were noted. The time the horses were not sufficiently sedated (i.e. HHAG $> 50\%$) was noted.

A paired t-test was used to compare the two treatments for normally distributed data (recovery time to 50% HHAG, recovery time to 100% HHAG). Ataxia VAS were analysed using a 2-way ANOVA (two within factors) for repeated measures followed by a Wilcoxon signed rank test whenever significance was detected. The level of significance was set at $p < 0.05$.

3.2.4. Results

Part I

There were no significant differences in romifidine requirements between the two treatments (R: 30 ± 7.5 ; RB: $29 \pm 7.0 \mu\text{g kg}^{-1}\text{hour}^{-1}$; $p = 0.74$). The time until the first additional bolus of romifidine was administered was significantly longer when butorphanol was added (R: 38.5 ± 13.6 minutes; RB: 50.5 ± 11.7 ; $p = 0.013$). There were no significant differences in romifidine requirements during the first hour including loading dose (R: 108 ± 14.0 ; RB: $100 \pm 13.3 \mu\text{g kg}^{-1}$; $p = 0.223$).

There were significant changes in ataxia score over time ($p < 0.001$) for both treatments but no significant differences between treatments ($p = 0.135$).

Shaking and trembling of head or muzzle was noticed in four horses while receiving R and in nine horses during RB ($p = 0.057$).

Overall, the horses urinated 30 times during R and 38 times during RB. The maximum number of times urination occurred was seven times during an individual study period (sedation until recovery).

Part II

Plasma concentrations were constant during the second hour of CRI (Fig. 5).

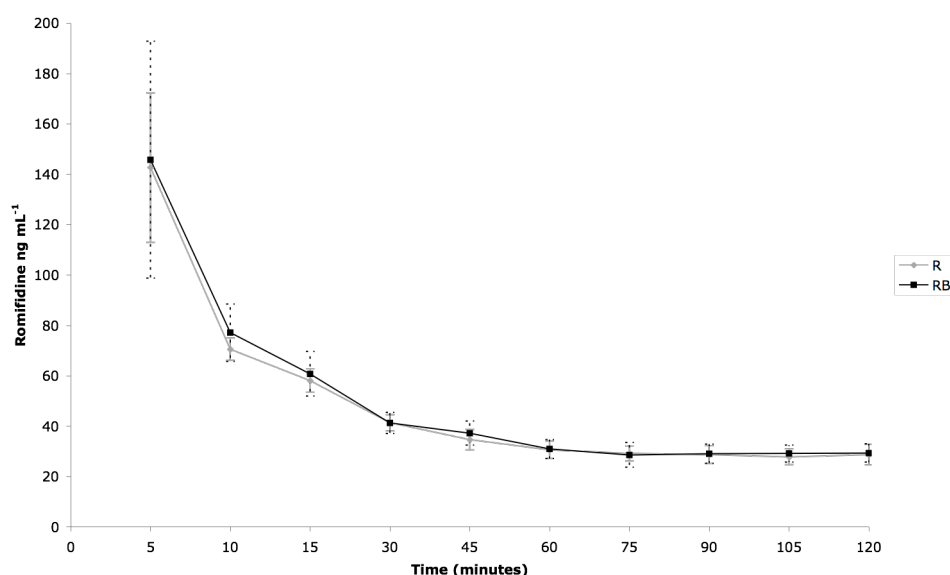


Figure 5: Plasma concentrations of romifidine (ng mL⁻¹) (mean \pm 95% CI) during a loading dose of romifidine 80 μ g kg⁻¹ (treatment R) or romifidine 80 μ g kg⁻¹ combined with butorphanol 18 μ g kg⁻¹ (treatment RB) IV followed by a constant rate infusion of romifidine at 30 μ g kg⁻¹ hour⁻¹ (treatment R) or romifidine 29 μ g kg⁻¹ hour⁻¹ combined with butorphanol 25 μ g kg⁻¹ hour⁻¹ (treatment RB) in 9 research horses.

One horse was excluded from the graphical representation because results of plasma concentrations did not follow the pattern observed in all the other horses. This was most likely due to an error in handling or collection of blood samples.

With both treatments all the horses remained sufficiently sedated until the end of the CRI (Fig. 6). Time to complete recovery (HHAG = 100%) was not significantly different between treatments (R: 59 ± 16.5 ; RB: 64.5 ± 19.9 minutes; $p = 0.371$).

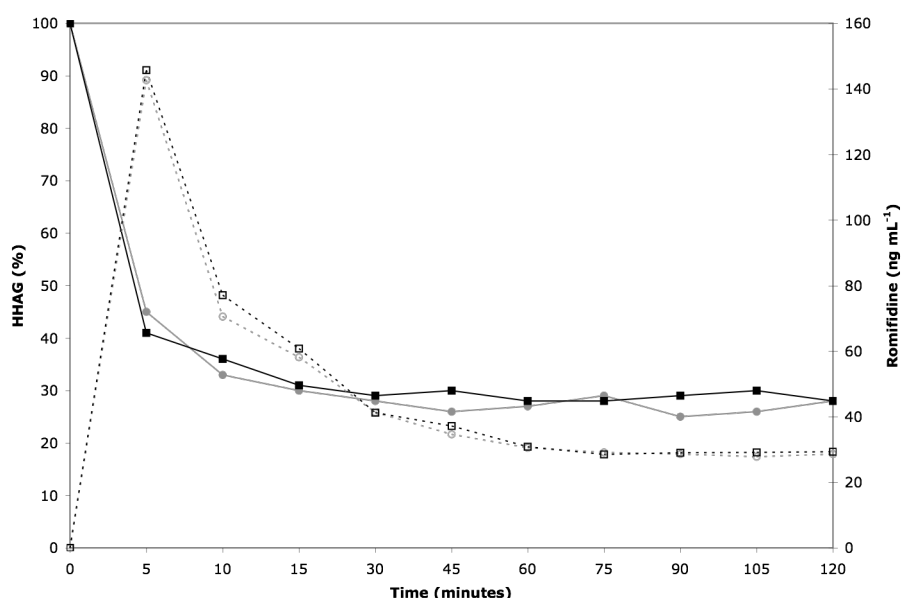


Figure 6: Head height above ground (HHAG) (mean \pm SD) (continuous lines) and romifidine plasma concentrations (mean \pm SD) (dotted lines) before and during administration of a loading dose of romifidine $80 \mu\text{g kg}^{-1}$ (treatment R) or romifidine $80 \mu\text{g kg}^{-1}$ combined with $18 \mu\text{g kg}^{-1}$ butorphanol (treatment RB) IV followed by a constant rate infusion of romifidine $30 \mu\text{g kg}^{-1} \text{ hour}^{-1}$ (treatment R); or romifidine $29 \mu\text{g kg}^{-1} \text{ hour}^{-1}$ combined with butorphanol $25 \mu\text{g kg}^{-1} \text{ hour}^{-1}$ (treatment RB) in 9 research horses.

Significant differences regarding degree of ataxia over time ($p < 0.001$) for both treatments and significant differences between treatments ($p = 0.039$) were detected: horses being significantly more ataxic when butorphanol was added (RB) (Fig. 7).

One horse fell to his carpal joints 30 minutes after starting the loading dose during treatment RB. He regained normal position immediately and remained in the study.

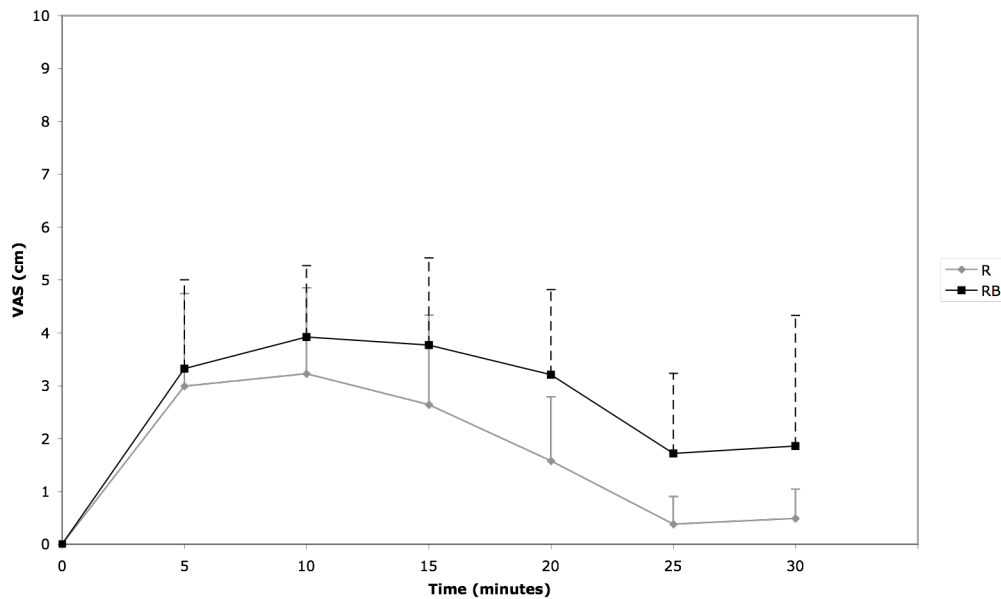


Figure 7: Ataxia assessed by a visual analogue scale (VAS) (0 cm meaning no ataxia and 10 cm maximal possible ataxia with the horse falling down) before and during loading doses of romifidine $80 \mu\text{g kg}^{-1}$ (treatment R) or romifidine $80 \mu\text{g kg}^{-1}$ combined with $18 \mu\text{g kg}^{-1}$ butorphanol (treatment RB) IV followed by a constant rate infusion of romifidine $30 \mu\text{g kg}^{-1} \text{ hour}^{-1}$ (treatment R); or romifidine $29 \mu\text{g kg}^{-1} \text{ hour}^{-1}$ combined with butorphanol $25 \mu\text{g kg}^{-1} \text{ hour}^{-1}$ (treatment RB) in 10 research horses. Mean \pm SD presented.

Horses demonstrated significantly ($p = 0.039$) higher ataxia scores when butorphanol was added (RB).

In total, the horses urinated 23 times during R and 29 times during RB. The maximal number of urinations observed per horse was 7.

3.2.5. Discussion

In the present study, CRI regimes of romifidine and romifidine combined with butorphanol that induce constant sedation in horses, as assessed by position of the head, were determined successfully. Although there was an initial effect of the loading dose of butorphanol on time to first additional romifidine requirements, over all, butorphanol did not reduce romifidine requirements over two hours of sedation. Constant romifidine plasma concentrations were achieved with both treatments.

A synergistic effect on sedation and analgesia has been associated with the addition of butorphanol to romifidine (Clarke et al. 1991; Kohler et al. 2004; Holopherne et al. 2005; DeRossi et al. 2009). However, in agreement with our results, other authors did not observe more profound sedation when butorphanol was administered with detomidine or romifidine (Clarke et al. 1991; Love et al. 2011). Also in a previous work with xylazine, no beneficial effect of butorphanol could be detected by using the same methods as in the present study

(Ringer et al. 2012a). The methods used may not be ideal to study the beneficial effects of adding butorphanol to an α_2 -adrenoceptor agonist. As the horses were unstimulated and pain free, mainly depth and not quality of sedation was tested in the present study. Therefore, the positive effect that butorphanol may have in clinical cases could have been missed. Nevertheless, the longer time until the first additional dose of romifidine observed with RB during part I, and the higher ataxia scores observed with RB during part II may be indicative of deeper sedation with butorphanol.

A prolonged sedative effect by adding butorphanol to romifidine has already been reported (DeRossi et al. 2009). In the present study, the horses receiving butorphanol did not require additional sedation until 50.5 ± 11.7 minutes (mean \pm SD) after the loading dose, compared to 38.5 ± 13.6 minutes (mean \pm SD) with R. This is similar to the 35 and 60 minutes of complete sedation observed by DeRossi et al. (2009) when using romifidine (0.1 mg kg^{-1}) or romifidine (0.1 mg kg^{-1}) plus butorphanol (0.05 mg kg^{-1}). Bolus doses of $0.020 - 0.050 \text{ mg kg}^{-1}$ of butorphanol have been effective in improving analgesia and sedation quality when combined with romifidine (Clarke et al. 1991; Kohler et al. 2004; Holopherne et al. 2005; DeRossi et al. 2009). Even with an initial effect of the loading dose of butorphanol on time to first romifidine re-dosing, there was no effect on the final dose rate ($\mu\text{g kg}^{-1} \text{ hour}^{-1}$) of romifidine. The dosing regime of butorphanol used in the present study has been shown to produce analgesia and constant plasma concentrations following colic surgery (Sellon et al. 2004). The effect of a higher constant rate infusion dose rate of butorphanol in the current model remains to be tested. There are no other studies investigating constant rate butorphanol infusions in combination with romifidine.

Ataxia, assessed by a VAS was consistently higher with RB (part I and II) and significantly higher during part II with RB, compared to R. With treatment RB, one horse fell to his carpal joints 30 minutes after receiving the loading dose. The horse did not appear severely ataxic before the event, and regained a normal stance immediately. However, an unacceptable degree of ataxia and/or falling over after α_2 -adrenoceptor agonist treatment (Freeman & England 2000), or after high doses of α_2 -adrenoceptor agonists with opioids (Paton & Clarke 1986; Clarke & Paton 1988; Greene & Thurmon 1988; Ringer et al. 2012a) has been observed previously. DeRossi et al. (2009) observed extreme ataxia in one horse when butorphanol was added to romifidine. During part I, ataxia scores were consistently higher with RB. However, they were not significantly different compared to R, probably because the

total dose of romifidine during the first 30 minutes was lower compared to part II where the CRIs were added. Over all, the degree of ataxia observed during the present protocols was considered to be acceptable.

The methods used in the present study are effective for the development of CRI regimes of α_2 -adrenoceptor agonists to provide constant sedation and plasma concentrations. This had been shown already with medetomidine and xylazine (Bettschart-Wolfensberger et al. 1999a; Ringer et al. 2012a). However, this was the first time the method was used with a longer acting α_2 -adrenoceptor agonist (England et al. 1992; England & Clarke 1996). No accumulation, defined as increasing plasma concentrations of romifidine, was observed after two hours of CRI. Long recoveries of up to 200 minutes and with an average duration of 160 minutes were observed after single doses of romifidine $80 \mu\text{g kg}^{-1}$ IV (England et al. 1992). Hamm et al. (1995) reported 90 minutes (mean duration) of head ptosis after $80 \mu\text{g kg}^{-1}$ romifidine IV. In the present study horses reached 100% HHAG 59 ± 16.5 and 64.5 ± 29.9 minutes (mean \pm SD) after discontinuing the CRIs of R and RB, respectively. The longest recoveries were 80 (R) and 95 (RB) minutes. Therefore, recovery seems to be faster after discontinuing a CRI compared to romifidine bolus administration. After reaching normal head position, sedation was not further evaluated, horses went back to pasture and no unusual behaviour was observed.

The time to maximal sedative effect (lowest HHAG) does not seem to coincide with maximal plasma concentrations of romifidine, reflecting a discrepancy between pharmacokinetics and pharmacodynamics. The delay between the IV administration of a drug and the onset of its clinical effect reflects the time necessary for the circulation to deliver the drug to its site of action (Stoelting & Hillier 2006). Therefore, cardiovascular effects produced by a drug might influence its time to clinical effect. Additionally, to exert their mechanism of action, the drug has to interact with a receptor and this will alter the function or conformation of a specific cellular component that initiates or prevents a series of changes that characterize the pharmacologic effect of the drug (Stoelting & Hillier 2006) and this will also delay the time to a clinical effect.

Clarke et al. (1991) observed muzzle tremors and head shaking when butorphanol was added to romifidine. These observations were interpreted as adverse effects of butorphanol. In the present study, there was a tendency of more frequent shaking/trembling of head/muzzles with RB compared to R, which was not statistically significant. However, occasional shaking and

trembling of the head or muzzle was observed with both treatments and can therefore not be attributed to the opioid only. The presentation of shaking/trembling of head or muzzles might also be related to depth of sedation rather than to opioid administration.

A diuretic effect is typical for α_2 -adrenoceptor agonists in horses (England & Clarke 1996). During a CRI of romifidine, independently of butorphanol, frequent urination was observed. Therefore, during prolonged sedation losses due to diuresis should be considered, especially if the horse is already debilitated or suffering from important fluid losses (e.g. bleeding).

The main limitation of the study regarding extrapolation to clinical practice is that healthy pain-free experimental horses were used. Differences regarding the effect of butorphanol on romifidine requirements and quality of sedation may exist in clinical cases where horses may be painful and stimulated. Also mainly Standardbred horses were included and the results should be extrapolated carefully to the whole equine population.

In the present study, protocols using romifidine with and without butorphanol providing constant sedation and plasma concentration have been successfully developed. The dose of butorphanol used in this study was ineffective in decreasing romifidine requirements. It is possible that higher CRI doses of butorphanol would have reduced romifidine requirements. Results should be extrapolated to clinical conditions with caution as data were obtained from healthy unstimulated research horses. Clinical efficacy studies will be necessary to show if clinical differences exist between the two protocols.

Appendix B: Analysis of romifidine plasma concentrations

Reagents and Chemicals - Romifidine was provided by Boehringer-Ingelheim France (Paris, France). Acetonitrile, HPLC grade, was obtained from Merck (Darmstadt, Germany), ammonium formate (mass spectroscopy grade) from Fluka (Steinheim, Germany). T-butyl methyl ether (TBME) was purchased from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany), KOH from Sigma-Aldrich (St. Louis, MO, USA).

Standard Preparation- An initial standard of romifidine was prepared by weighing 10 mg of the standard and then dissolving it in acetonitrile to get the exact volume of 100 mL. The solution was kept in a freezer at -18°C for no more than a month. It was used, after letting it reach room temperature for about one hour, to prepare diluted standards as needed. 100 µg mL⁻¹, 10 µg mL⁻¹, 1 µg mL⁻¹ and 0.1 µg mL⁻¹ working standard solutions were prepared by dilution in acetonitrile and were used as spiking solutions. A 1 µg mL⁻¹ solution in a mixture of acetonitrile and ammonium formate 10 mM (50:50) was prepared for infusion in the ESI source to optimize the MS conditions.

Samples preparation- Extraction was adapted from the method described by Hammer et al. 2004.

Five mL of plasma were introduced in a 15 mL tube. To adjust the pH to 14, 1.2 mL KOH (1 M) were added. Afterwards 5 mL of TBME were added. The obtained solution was horizontally shaken during 5 minutes and afterwards centrifuged at 3000 rpm during 5 minutes. Afterwards the upper phase was transferred to a glass tube and the TBME evaporated to dryness at 40°C under a gentle stream of nitrogen. The residues were reconstituted by 100 µL of mobile phase and transferred into a glass vial for injection in the LC-MS system.

LC-ESI/MS- The LC-ESI/MS used was a 1100 Series LC/MSD ion Trap VL with an ElectroSpray Ionisation (ESI) interface and a LCMS Chemstation software from Agilent Technologies (Palo Alto, CA, USA).

Chromatographic separation was performed using a Zorbax Eclipse-C18 (2.1 mm × 10 mm, 3.5 µm) column and a Zorbax Eclipse XDB-C8 (2.1 mm × 12.5 mm, 5µm) guard-column from Agilent Technologies (Palo Alto, CA, USA) with a mobile phase of A: 10 mM ammonium formate, 0.1% formic acid and B: acetonitrile HPLC grade. The mobile phase gradient elution was 80%A:20%B (v:v) at 0 minutes, increasing to 50%A:50%B from 0 to 5 minutes, holding at 80%B from 6 to 7 minutes and back at 20% B at 8 minutes for a total run time of 18 minutes including column equilibration. The column temperature was 30°C. The flow rate in the LC column was 0.25 mL minute⁻¹. The injection volume was 1µL. The temperature of the autosampler tray was set to 5°C.

Detection was by MS with electrospray ionization in positive mode. Nebulizer pressure was set to 40 psi, dry gas temperature to 350°C, and dry gas flow to 8 L minute⁻¹. On the basis of infusion experiments, optimized MS instrument conditions were determined. Capillary voltage was set to 3500V, CID to 1V. Collision gas in the trap was helium with a pressure of 0.6*10⁻⁵ mbar.

Validation of the LC-MS method

Identification- The identification criteria for romifidine were the retention time (t_r = 2.1 minutes) and the ions (m/z (+)=258;260) due to bromine isotopes.

Specificity- Specificity was tested with blank horse plasma (10 probes) and there were no interferences to be found.

Limit of quantification- Considering the mean signal to noise ratio $S/B_{(blank)}$ and the standard deviation $\sigma_{(blank)}$ for 10 blank injections, the limit of quantification (LOQ) was determined by the mean of 5 blank samples

spiked in such a way we obtained a signal intensity at least equal to $S/B_{(\text{blank})} + 10\sigma_{(\text{blank})}$. The LOQ was 0.4 ng mL⁻¹.

Linearity- Linearity was tested from 2 to 200 ng mL⁻¹. Four replicates of spiked blank horse plasma spiked at six different concentrations were performed for a total of 24 injections. The response was linear throughout the concentration range tested with the coefficient of correlation (r^2) equal to 0.997, and the calibration curve obtained was $A_{\text{romifidine}} = 64024.01 * C_{\text{romifidine}} + 62927.05$. The peak areas ($A_{\text{romifidine}}$) were then used in conjunction with the calibration curve to determine the concentration of romifidine ($C_{\text{romifidine}}$) in ng mL⁻¹ in the samples.

Precision- The precision was evaluated by performing four replicates of six spiked quality control (QC) samples. The mean intra-day accuracy ($n = 4$) for each concentration tested was between 90 and 114%, except for the LOQ (2 ng mL⁻¹) where the accuracy was 75%.

Matrix effects- While infusing 100 ng mL⁻¹ romifidine solution, a blank horse plasma was injected and no matrix effects were observed.

The extraction rate was tested and evaluated to 85%.

3.3 Trial 3: The effects of a loading dose followed by constant rate infusion of xylazine compared with romifidine on sedation, ataxia and response to stimuli in horses

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3.3.1. Summary

Objective: To compare xylazine and romifidine constant rate infusion (CRI) protocols regarding degree of sedation, and effects on postural instability (PI), ataxia during motion (A) and reaction to different stimuli.

Study design: Blinded randomized experimental study

Animals: Ten adult research horses

Methods: Degree of sedation was assessed by head height above ground (HHAG). Effects on PI, A and reaction to visual, tactile and acoustic stimulation were assessed by numerical rating scale (NRS) and by visual analogue scale (VAS). After baseline measurements, horses were sedated by intravenous loading doses of xylazine (1 mg kg⁻¹) or romifidine (80 µg kg⁻¹) administered over 3 minutes, immediately followed by a CRI of xylazine (0.69 mg kg⁻¹hour⁻¹) or romifidine (30 µg kg⁻¹ h⁻¹) which was administered during 2 hours. Degree of sedation, PI, A and reaction to the different stimuli were measured at different time points before, during and for one hour after discontinuing drug administration.

Data were analysed using two-way repeated measures ANOVA, a Generalized Linear Model and a Wilcoxon Signed Rank Test ($p < 0.05$).

Results: Significant changes over time were seen for all variables. With xylazine HHAG was significantly lower 10 minutes after loading dose, and significantly higher at 150 and 180 minutes compared to romifidine. Reaction to acoustic stimulation was significantly more pronounced with xylazine. Reaction to visual stimulation was greater with xylazine at 145 and 175 minutes only with VAS. PI was consistently but not significantly greater with xylazine during the first 30 minutes. Reaction to touch and A were not different between treatments.

Conclusions: Time to maximal sedation and recovery is longer with romifidine. However, horses seem to be more arousable with xylazine.

Clinical relevance: With romifidine sufficient time should be allowed for complete sedation before manipulation. The two protocols should be compared under clinical conditions.

3.3.2. Introduction

Many diagnostic and minor surgical procedures can be performed on the standing sedated horse. However a reliable sedation protocol is essential. The protocol should provide sufficient degree and duration of sedation, good analgesia, no or minimal reaction to external stimuli, and produce minimal ataxia. Another desirable feature is that the drugs used are registered for horses.

Due to their characteristics, α_2 -adrenergic agonists or their combinations are frequently used for this purpose. For prolonged sedation the use of α_2 -adrenergic agonists as a CRI seems to be associated with less adverse effects and to be less cumbersome than repeated boli administration (Bettschart-Wolfensberger et al. 1999b; Ringer et al. 2012a, 2012b). Additionally, duration of sedation can be prolonged as required. However when used as a CRI, the different α_2 -adrenergic agonists have never been compared regarding degree of sedation, ataxia and reaction to external stimuli.

The α_2 -adrenergic agonists xylazine and romifidine are both registered for horses and CRI protocols providing prolonged sedation and constant plasma concentrations have been developed previously (Ringer et al. 2012a, 2012b). Romifidine has been reported to produce less ataxia compared to detomidine and xylazine when a single, equi-sedative dose was used (England et al. 1992; Hamm et al. 1995). However, compared to romifidine, xylazine seems to have fewer effects on cardiovascular function when used as a loading dose followed by a CRI (Ringer et al. unpublished data).

The goal of the present study was to compare xylazine and romifidine loading doses followed by CRIs regarding degree of sedation, ataxia, and reaction to tactile, visual and auditory stimulation in research horses.

3.3.3. Materials and Methods

This study was approved by the Ethical Committee of the National Veterinary School of Lyon (N°0807, May 13th 2008).

Ten healthy adult research horses (4 geldings, 6 mares) of different breeds (8 French Standardbreds, 1 Thoroughbred, 1 French Saddlebred) with an age of 9 ± 2.4 years (mean \pm SD) (range 6 – 14 years) and a body weight of 530 ± 49.1 kg (range 430 – 600 kg) were included in the study. During the trial, the horses were maintained in groups on pasture with free access to hay and water. The night before sedation, the region of the jugular vein was clipped and the horses were stabled individually for habituation.

The morning of the experiment, rectal temperature, heart rate and respiratory rate were measured, heart and lungs were auscultated, and mucous membranes and skin turgor evaluated. Subsequently, a 14 gauge x 160 mm catheter (SecalonT; Ohmeda, UK) was placed into one of the jugular veins under local anaesthesia with subcutaneous lidocaine (Xylovet, CEVA Santé Animale, France). After catheter placement, the horses were tied up close to a scale that had been previously marked on the wall (Fig. 8). The upper limit of the scale was marked as 10 and was situated at 128 cm from the floor. The distance to the floor was then divided into ten equal parts representing a 0 (the floor) – 10 scale.

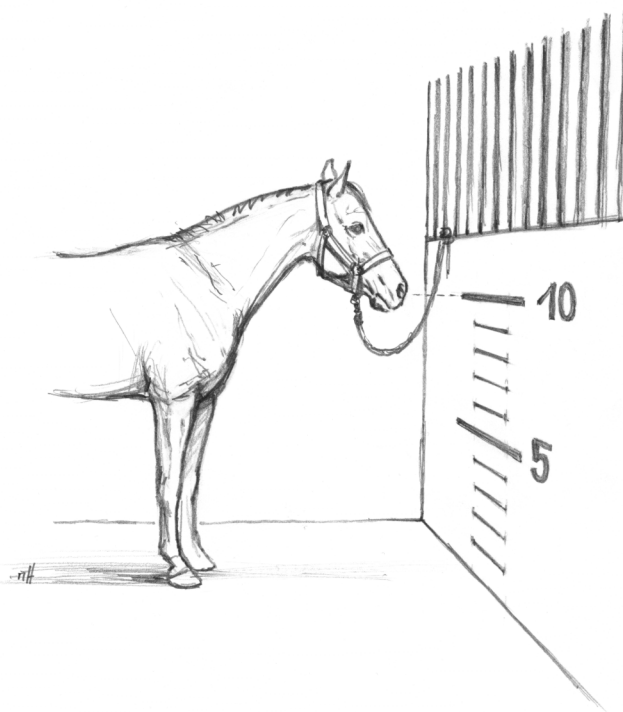


Figure 8: Depth of sedation was assessed by using the head height above the ground (HHAG). The HHAG was defined as the position of the nose in relation to a scale marked on the wall. The horse on the image shows a HHAG of 10.

After one hour of habituation baseline values for different variables were assessed. Degree of sedation was assessed by using the head height above ground (HHAG), which was measured by looking at the position of the nose related to the scale previously marked on the wall (Fig. 8). Ataxia was scored by looking at the horse while standing (postural instability = PI) and while walking in a straight line approximately 20 m, then turning and walking back (ataxia during motion = A). Postural instability and A were scored by a numerical rating scale (NRS) (Table 4) and by a visual analogue scale (VAS).

Table 4: Numerical rating scales (NRS) used to assess postural instability, ataxia during motion, and response to tactile, visual and acoustic stimulation in horses.

NRS	Postural Instability	Ataxia during motion	Touch	Visual	Acoustic
0	No signs of instability, stable	No signs of instability	Exaggerated reaction after smooth pressing: fast moving of the leg	Undiminished response, animal moves away vigorously	Undiminished response, animal turns vigorously
1	Stable but swaying slightly	No ataxia when straight, slight ataxia when turning	Animal elevates the front leg after normal pressure	Muted response, subdued reaction and movements	Muted response, subdued reaction and movements (turns slowly)
2	Swaying clearly	Ataxia when walking straight	Slightly diminished response with normal to strong pressing	Reaction significantly subdued (elevates head slightly)	No appreciable response, but evidence of hearing (movement of ears)
3	Nearly falling down	Severe ataxia, risk of falling down	No response even with strong pressing	No signs of visual arousal	No sign of noise recognition
4	Horse falling down	Horse falling down			

The VAS consisted in a 10 cm line with 0 cm representing no ataxia and 10 cm representing a maximal possible ataxia with the horse falling down. Response to touch was tested by using a pole with a blunt nail at the end. The nail was gently pressed against the coronary band of the thoracic and pelvic limb and reaction assessed. The order of stimulation was alternated, once

starting with the pelvic and next time with the thoracic limb. Visual stimulation was performed by opening an umbrella in front of the horse. Response to acoustic stimulation was assessed using a recorded horse nicker and a metallic noise produced with a tin and a spoon. Reactions to tactile, visual and acoustic stimulation were assessed using a NRS (Table 4) and a VAS.

The VAS used consisted in a 10 cm line where 0 cm represents no reaction and 10 cm the maximal possible reaction.

It was a blinded, randomized, crossover study and all the observations were done by a single observer.

After baseline measurements horses were sedated with a loading dose xylazine (1 mg kg^{-1}) (Xylasol ad us. vet., Dr. E. Graeub AG, Switzerland) or romifidine ($80 \text{ } \mu\text{g kg}^{-1}$) (Sedivet®, Boehringer Ingelheim France, Paris, France) injected manually over 3 minutes through the jugular venous catheter. Immediately afterwards a CRI of xylazine ($0.69 \text{ mg kg}^{-1} \text{ hour}$) or romifidine ($30 \text{ } \mu\text{g kg}^{-1} \text{ hour}$) was started for 2 hours. The infusions were delivered through extension sets (Original Perfusor-Leitung PE, B. Braun AG, Germany) by an infusion pump (Syramed® μSP6000 , Arcomed AG, Switzerland) situated outside of the box stable. Romifidine was diluted into 0.9% saline (Chlorure de Sodium 0.9% B. Braun, B. Braun Medical, Boulogne Cedex, France) so that equal volumes could be used with both treatments for loading dose and CRI.

The different variables were measured at different time-points and not all of them at once, in order to avoid stimulation of one type to influence response to a different stimulation (Table 5).

Table 5: Head height above the ground (H), postural instability (PI), ataxia during motion (A), and reaction to tactile (T), visual (V) and acoustic (Ac) stimulation were assessed at different time-points before (B), during (0 – 120 minutes) and after (125 -185 minutes) α_2 -adrenergic agonist administration.

Time (minutes)	Stimuli					
	H	PI	A	T	V	Ac
B	x	x	x	x	x	x
10	x	x				
15						
20	x	x		x		
25					x	
30	x	x				
35						x
40	x	x				
45						
50				x		
55					x	
60	x	x	x			
65						x
70						
75						
80				x		
85					x	
90	x	x	x			
95						x
100						
105						
110				x		
115					x	
120	x	x	x			
125						x
130						
135						
140				x		
145					x	
150	x	x	x			
155						x
160						
165						
170				x		
175					x	
180	x	x	x			
185						x

Ataxia during motion was not assessed during the first hour after loading dose application to avoid accidents due to exaggerated ataxia.

Frequency of urination and of snoring was noted.

Statistical analyses were performed using the software packages SigmaStat[®] 3.5 (SigmaStat[®] 3.5, Systat Software GmbH, Ekrath, Germany) and R (R development Core Team 2012). Normality was tested by plotting data graphically and by using the Kolomogrov-Smirnov normality test.

A two way repeated measures ANOVA followed by a generalized linear mixed model was used to study the differences between treatments and over time. A Holm-Sidak versus control (B) was used as post hoc test. Whenever significance was detected, a Wilcoxon Signed Rank test was used as confirmation.

A Wilcoxon Signed Rank Test was used to compare the two treatments regarding frequency of urination.

Significance was set at $P < 0.05$.

3.3.4. Results

The experiments were performed in the wintertime in order to avoid disturbance of horses due to insects. One horse had to be excluded because the alpha₂-adrenergic agonist infusion was not running for 10 minutes because the line was frozen.

With xylazine the HHAG was significantly lower 10 minutes ($p = 0.008$) after starting loading dose administration and higher at 150 ($p = 0.004$) and 180 minutes ($p = 0.004$) compared to romifidine (Fig. 9a).

There was no significant difference regarding HHAG between treatments from 20 to 120 minutes after starting alpha₂-adrenergic agonists. With xylazine, 30 minutes after stopping CRI (i.e. 150 minutes) there was no significant difference in HHAG compared to baseline values. With romifidine, one hour after stopping CRI, HHAG did not return to baseline values.

Significant changes in PI were observed over time with VAS and NRS (Fig. 9b and Table 6). Graphically, NRS and VAS were consistently higher with xylazine, however not significantly different from romifidine (Fig. 9b).

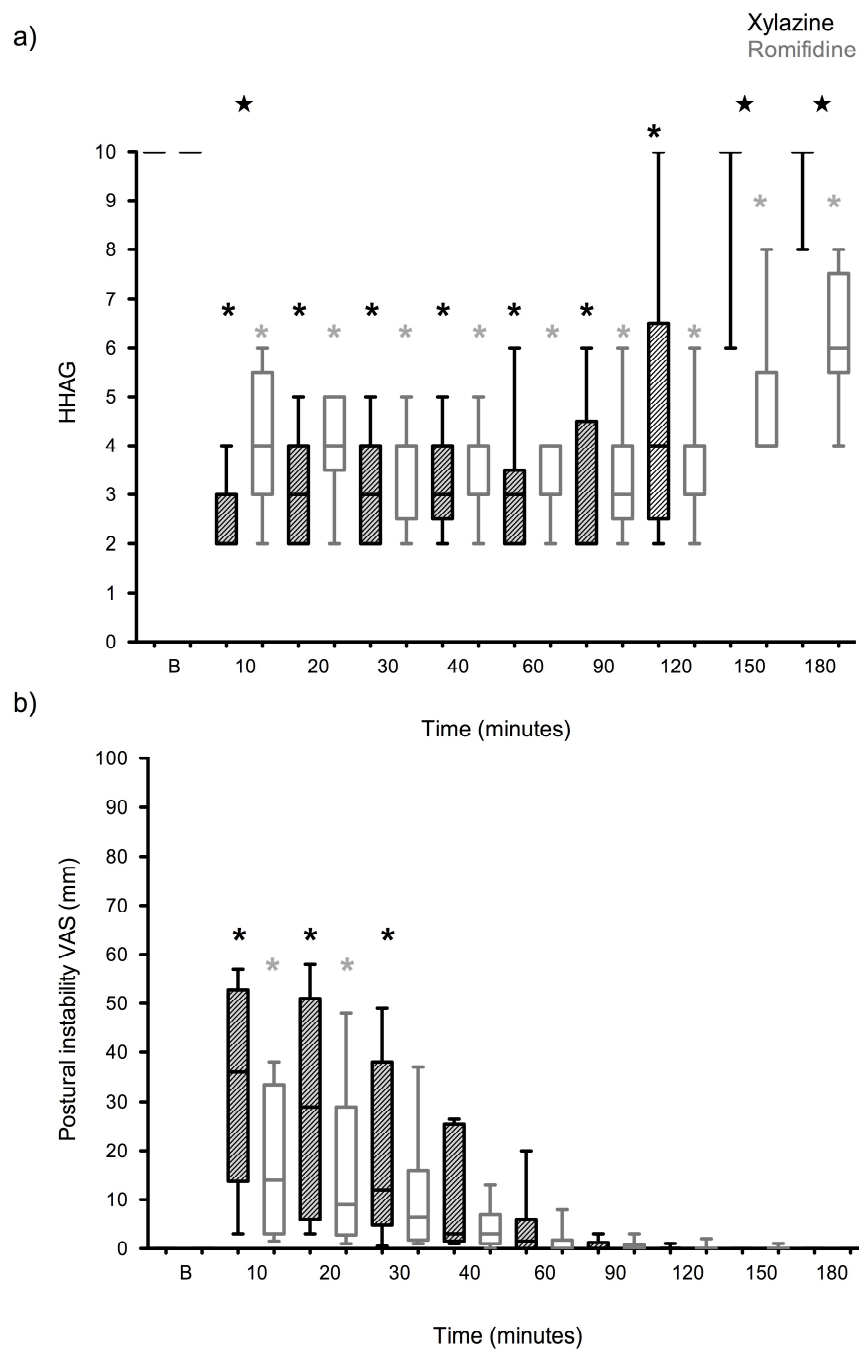


Figure 9: Head height above ground (HHAG) (a) and Postural instability (b) assessed by a visual analogue scale (VAS) in nine horses. Measurements were recorded before (B), during drug administration (intravenous loading dose of xylazine 1 mg kg⁻¹ or romifidine 80 mcg kg⁻¹; followed by two hours of constant rate infusion (CRI) of xylazine 0.69 mg kg⁻¹ hour⁻¹ or romifidine 30 mcg kg⁻¹ hour⁻¹) (time points 0 – 120 minutes) and during one hour after discontinuing CRI (125 – 185 minutes).

* Significant difference (P < 0.05) compared to the baseline (B) of the same treatment

*Significant difference (P < 0.05) between treatments

Table 6: Postural instability, ataxia during motion, and reaction to visual, tactile and acoustic stimulation assessed by numerical rating scale (NRS) in nine horses (median [range]). Reaction to touch was assessed on both the thoracic and pelvic limb. For acoustic stimulation horse nicker and a metallic noise was used. Measurements were recorded before (Baseline), during drug administration (intravenous loading dose of xylazine 1 mg kg⁻¹ or romifidine 80 mcg kg⁻¹; followed by two hours of constant rate infusion (CRI) of xylazine 0.69 mg kg⁻¹ hour⁻¹ or romifidine 30 mcg kg⁻¹ hour⁻¹) (time points 0 – 120 minutes) and during one hour after discontinuing CRIs (125 – 185 minutes).

* Significant difference (P < 0.05) compared to the baseline (B) of the same treatment

*Significant difference (P < 0.05) between treatment

Time (minutes)										
Postural instability										
	Baseline	10	20	30	40	60	90	120	150	180
X	0(0-0)	2(1-2)*	1(1-2)*	1(1-2)*	1(0-1)*	1(0-1)*	0(0-1)	0(0-0)	0(0-0)	0(0-0)
R	0(0-0)	1(1-2)*	1(1-2)*	1(1-1)*	1(0-1)*	0(0-1)	0(0-1)	0(0-0)	0(0-0)	0(0-0)
Ataxia during motion										
	Baseline					60	90	120	150	180
X	0(0)					2(0-2)*	0(0-2)*	0(0-1)*	0(0)	0(0)
R	0(0)					2(1-2)*	1(0-2)*	0(0-1)*	0(0)	0(0)
Response to touch thoracic limb										
	Baseline	20		50		80	110	140		170
X	1(0-3)	3(0-3)*		2(0-3)*		2(0-3)	1(0-3)*	2(0-2)		1(0-3)
R	1(0-3)	2(0-3)*		2(0-3)*		2(0-3)	3(0-3)*	2(0-3)		2(0-3)
Response to touch pelvic limb										
	Baseline	20		50		80	110	140		170
X	1(0-2)	1(0-3)*		2(0-3)*		1(0-3)*	2(0-3)*	1(0-3)		1(0-3)*
R	1(0-2)	3(0-3)*		2(0-3)*		2(0-3)*	2(0-3)*	2(0-3)		1(0-3)*
Response to visual stimulation										
	Baseline	25		55		85	115	145		175
X	0(0-1)	1(0-2)*		1(0-3)*		2(0-2)*	1(0-2)*	0(0-1)*		0(0-1)
R	0(0)	1(0-2)*		2(0-3)*		2(0-2)*	1(0-2)*	1(0-3)*		0(0-2)
Response to acoustic stimulation by horse nicker										
	Baseline		35		65	95	125	155		185
X	0(0-1)		1(0-2)*		2(0-2)*	2(0-2)*	2(0-2)*	0(0-2) *		0(0) *
R	0(0-1)		2(1-2)*		2(1-2)*	2(1-2)*	2(1-2)*	2(1-2)*		2(1-2)*
Response to acoustic stimulation by metallic noise										
	Baseline		35		65	95	125	155		185
X	1(0-2) *		1(0-2)*.*		1(0-2)*.*	1(0-2)*.*	1(0-2)*.*	1(0-2)*.*		1(0-2)*.*
R	1(0-2)		2(1-3)*		2(1-2)*	2(1-3)*	2(1-3)*	2(1-2)*		2(1-2)*

There were no significant differences in A between the two treatments with neither score (VAS or NRS). However there were significant changes over time with both treatments for NRS ($p < 0.001$) and VAS ($p < 0.001$) (Fig. 10a and Table 6).

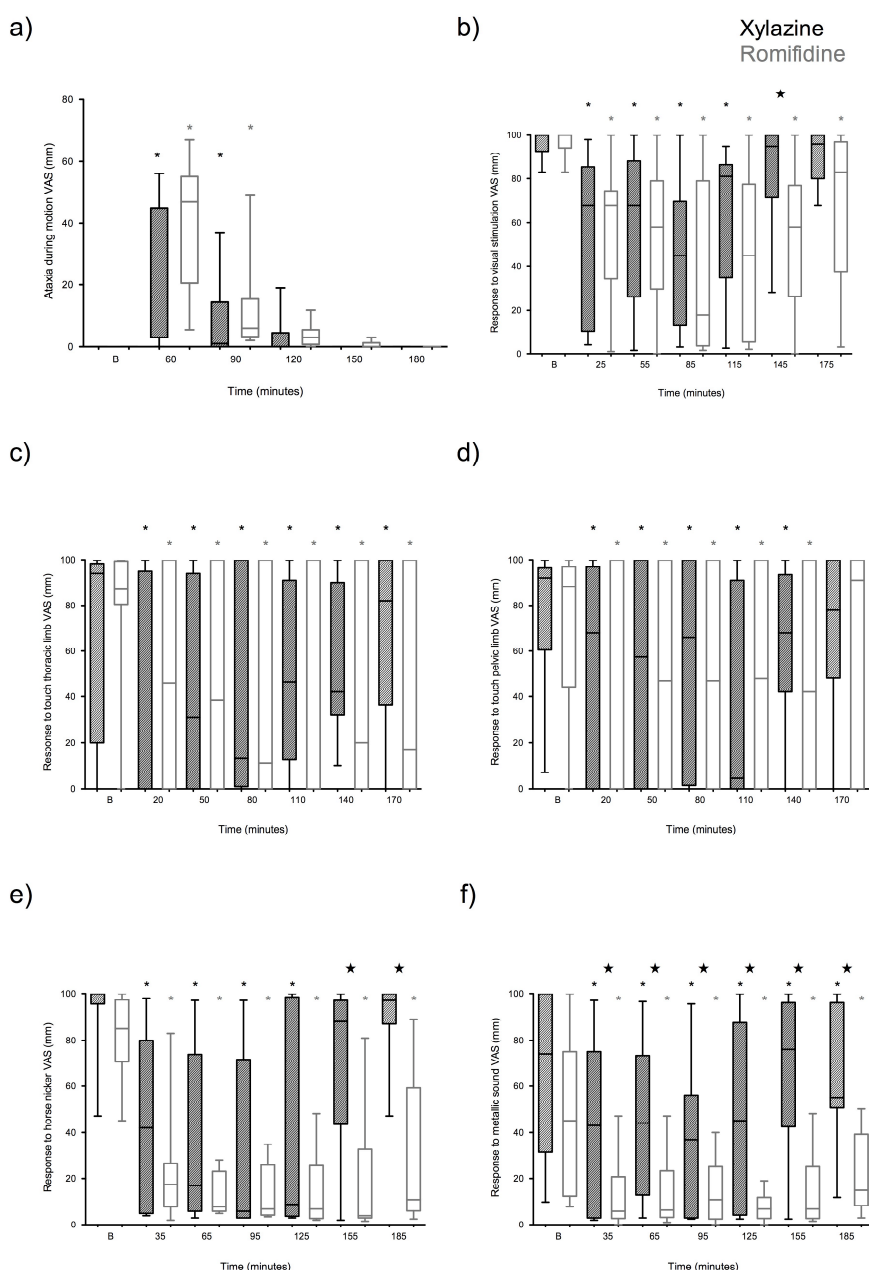


Figure 10: Ataxia during motion (a), and reaction to visual (b), tactile (c,d) and acoustic (e,f) stimulation assessed by visual analogue scale (VAS) in nine horses. Reaction to touch was assessed on both, the thoracic (c) and pelvic limb (d). For acoustic stimulation horse nicker (e) and a metallic noise (f) was used. Measurements were recorded before (B), during drug administration (intravenous loading dose of xylazine 1 mg kg^{-1} or romifidine 80 mcg kg^{-1} ; followed by two hours of constant rate infusion (CRI) of xylazine $0.69 \text{ mg kg}^{-1} \text{ hour}^{-1}$ or romifidine $30 \text{ mcg kg}^{-1} \text{ hour}^{-1}$) (time points 0 – 120 minutes) and during one hour after discontinuing CRIs (125 – 185 minutes).

* Significant difference ($P < 0.05$) compared to the baseline (B) of the same treatment

**Significant difference ($P < 0.05$) between treatments

No significant differences between treatments were observed while looking at reaction to touch (Fig. 10c, d and Table 6). However, a significant decrease in reaction to touch compared to baseline could be observed with the VAS and NRS on both the thoracic and pelvic limb after xylazine and romifidine administration.

Regarding visual stimulation there were significant changes over time compared to baseline with both treatments for VAS and NRS (Fig. 10b and Table 6). By using the NRS no significant differences between treatments were detected. With VAS, reaction to visual stimulation was significantly more intensive with xylazine at 145 minutes ($p = 0.008$), and there was a strong tendency ($p = 0.055$) at 175 minutes.

With acoustic stimulation by horse nicker significant changes compared to baseline were seen with both treatments and scoring systems (Fig. 10e and table 6). Treatments were only significantly different at the last two measurements, meaning at 155 and 185 minutes with both VAS ($p = 0.02$; $p = 0.008$) and NRS ($p = 0.031$; $p = 0.004$).

A significantly increased reaction to metallic noise was seen with the NRS ($p < 0.001$) and VAS ($p = 0.001$) for xylazine compared to romifidine (Fig. 10f and table 6). Also changes over time were seen for the NRS ($p = 0.002$) and VAS ($p < 0.001$) with both treatments.

With romifidine, horses urinated significantly more frequently ($p = 0.047$) than with xylazine. With romifidine horses urinated a median of 4 times (range: 1 - 6) and with xylazine 2 (range: 0 - 3) during the observation period, starting from α_2 -adrenergic agonist administration.

With both treatments five out of nine horses snored.

3.3.5. Discussion

Based on HHAG, both protocols provided equal degree of sedations from 20 minutes after starting loading doses until the end of CRI. No significant differences between α_2 -adrenergic agonists were observed regarding PI, A or reaction to tactile stimulation. A more pronounced reaction to metallic noise was observed with xylazine compared to romifidine. With xylazine reaction to visual stimulation and to horse noise during the recovery period started earlier.

The loading doses used in the present study have been described as being equipotent regarding sedation (England et al. 1992). The CRI dosages have been developed using exactly the same materials and methods and have been shown to provide constant plasma concentrations (Ringer et al. 2012a, 2012b).

The head height above the ground (HHAG) has been frequently used to objectively assess degree of sedation provided by α_2 -adrenergic agonists (Bryant et al. 1991; Clarke et al. 1991; England et al. 1992; Hamm et al. 1995; Freeman & England 1999, 2000; Figueiredo et al. 2005; Nannarone et al. 2007). Advantages of the HHAG are that it is a quantitative and objective variable, and that the horses do not need to be stimulated for measurement. A lowering of the head results from muscle relaxation, sedation and reduced awareness (Freeman & England 1999; Figueiredo et al. 2005). It might be difficult to differentiate degree of sedation from quality of sedation in unstimulated horses. Actually, HHAG might be more indicative for degree than for quality of sedation (Hamm et al. 1995). Therefore in the present study we included different parameters in addition to the HHAG to further assess quality of sedation. The scores used in the present study have been partially modified from Hamm et al. (1995).

This is the first time two α_2 -adrenergic agonists are compared experimentally regarding degree and quality of sedation when administered as a CRI. First results of a clinical study comparing romifidine and detomidine CRIs have been presented recently (Nannarone et al. 2011).

Based on HHAG degree of sedation was equal between the two treatments from 20 minutes after starting loading doses until the end of drug administration (Fig. 9a). This does not agree with the studies performed by England et al. (1992) where lowering of the head was less pronounced with romifidine than with xylazine when single considered equipotent doses were used. However also in the present study, HHAG was significantly lower with xylazine compared to romifidine 10 minutes after starting the loading dose. This can be attributed to a faster action compared to romifidine, or due to a transiently more profound sedation with xylazine at 10 minutes after starting loading dose administration. The latter would better agree with the fact that the lowest median value observed in Fig. 9b correspond to xylazine at 10 minutes. However, when looking at the HHAG of the individual horses, in five horses the lowest value observed with xylazine was not different compared to the lowest observed with

romifidine. In another four horses the lowest recorded HHAG was lower with xylazine compared to romifidine.

The significantly lower head position observed with romifidine at 30 (150 minutes) and 60 (180 minutes) minutes after stopping CRI is probably due to a longer action of romifidine compared to xylazine. One hour after stopping romifidine CRI, HHAG remained significantly lower compared to baseline indicating residual sedation. The dose-dependent prolonged sedative effect of romifidine compared to xylazine has already been described (England et al. 1992). The prolonged effect that has been observed with romifidine might be due to the reservoir of romifidine in the corpuscular fraction of blood and its slow release in blood (Romagnoli et al. 2009).

No significant difference between treatments could be detected in PI or A. However, there was tendency to more PI with xylazine during the first 20 minutes after starting loading dose administration (Fig. 9b). This tendency to more severe PI with xylazine might have become significant with a higher number of horses. However the increased PI occurs principally during the first 20 minutes after starting loading dose administration, corresponding to a lower HHAG (Fig. 9) and therefore might be also attributed to transiently deeper sedation with xylazine. Contrary to our results, previous studies showed that instability and ataxia were significantly less with romifidine compared to other α_2 -adrenergic agonists (England et al. 1992; Hamm et al. 1995; Nannarone et al. 2007). However, it might be possible that during CRI, horses do learn to counterbalance the instability making differences between treatments less evident over time in the present study. On the other hand also the different methods used in the different studies to evaluate ataxia might have influenced the results.

Graphically overall reaction to visual stimulation was consistently lower with romifidine compared to xylazine (Fig. 10b). However significant differences between treatments were only measured after discontinuing CRIs. This is probably related to the longer effect or residual sedation of romifidine. With both treatments and with both scoring systems, significant changes over time were seen with a decreased reaction after and during α_2 -adrenergic agonists administration and a returning to baseline at the end of the observation period (Fig. 10b and table 6). However with romifidine, response remained significantly decreased for one hour after discontinuing drug administration, where observation ended. A decreased reaction to audiovisual stimulation after α_2 -adrenergic agonists has been reported before (Clarke et al. 1991), however this has not been studied during CRI.

No significant differences regarding reaction to touch were observed between treatments. Baseline scores were very variable depending on the horse, some of them showing already exaggerated reaction (score 0; Vas 100) and others no response even with strong pressing (score 3; VAS 0) (Fig. 10c,d and Table 6). In general those horses kept their reaction pattern during α_2 -adrenergic agonist administration. Therefore, reaction to touch seems to be dependent on the individual horse more than on treatment. Some horses had exaggerated reaction independently of α_2 -adrenergic agonist sedation and others had no reaction. However, an overall significant decrease in reaction after starting α_2 -adrenergic agonists could be observed with both scoring systems, on both extremities and with both treatments (Fig. 10c,d and table 6). An increased reaction to touch was not observed on the thoracic or pelvic limb after administering α_2 -adrenergic agonists or during CRI. Reaction to touch despite heavy sedation has been reported when α_2 -adrenergic agonists are administered alone (England & Clarke 1996), and this could be confirmed by our data for individual horses. Some horses showed increased reaction compared to baseline after stopping CRI, and this might be due to a learning effect in face of a weaning sedation. The learning effect remained despite efforts to minimize it by alternating the order of limb stimulation. Some horses reacted when the pole was only approximating, before touching the skin. Stimulation devices with remote control that do not need approximation to the patient would have been a better choice (Moens et al. 2003). Nevertheless we consider our model useful, as also under clinical circumstances the surgeon has to manipulate certain body parts of the horse and certain horses will also learn which action is followed by an unpleasant feeling and react concomitantly.

With both treatments there was a significant effect of α_2 -adrenergic agonists on reaction to acoustic stimulation. The effect on reaction to metallic noise is overall greater with romifidine compared to xylazine. The decreased reaction to metallic noise could be a characteristic of romifidine itself or that horses were generally more arousable with xylazine. However, other types of stimulation could not confirm the latter. Regarding stimulation by horse nicker, during drug administration there was no significant difference between treatments. However, after stopping CRIs the effect on reaction to acoustic stimulation by horse nicker is prolonged with romifidine compared to xylazine, probably due to the prolonged effect of romifidine.

An increased diuretic effect due to α_2 -adrenergic agonists has been reported before (England & Clarke 1996). The increased diuretic effect of romifidine compared to xylazine has never been reported. The increased frequency of urination with romifidine can be due to a more prolonged effect of romifidine or because of a more potent or different diuretic effect. A difference in diuretic response between xylazine and medetomidine has been reported in dogs (Talukder & Hikasa 2009). This difference was attributed to different selectivity and specificity to α_2 -adrenoceptors and/or imidazoline receptors. The difference on diuresis between xylazine and romifidine in horses could probably also be attributed to these differences in specificity and selectivity to α_2 -adrenergic and imidazoline receptors. Different mechanisms leading to diuresis due to α_2 -adrenergic agonists have been discussed, however the exact mechanism remains unknown and is probably a result of multiple haemodynamic, neural, hormonal and local factors in the kidney (Talukder & Hikasa 2009).

Snoring during sedation has been reported with α_2 -adrenergic agonists and has been attributed to gravitational effects because of low head position and to the muscle relaxant effects of α_2 -adrenergic agonists (England & Clarke 1996). No complications were observed due to this partial upper airway obstruction. However if the CRIs are used over a prolonged time period, horses should be monitored for upper airway obstruction. The head should be supported to reduce swelling of the nasal mucosa.

The main limitations of the study are that even by using multiple parameters to assess quality of sedation, under experimental conditions it is very difficult to mimic a clinical situation. Therefore, controlled clinical studies are warranted.

The use of scores and their following statistical analysis has been frequently discussed. In the present study, as there is no established scoring system for horses we decided to use both a NRS and a VAS. However, based on our experience and agreeing with Freeman et al. (1999) we do consider the VAS more appropriate, at least for an experienced observer, to assess the different parameters. However, in the present study the observer was blinded to treatment, but not to measurement point and this might have led to bias regarding changes over time.

Frequently scores are not normally distributed and they should not be treated as parametric data. In our study we were interested in comparing the two treatments and the effect of time. Therefore, the perfect test would have been a non-parametric two way repeated measures ANOVA, which unfortunately does not exist. In order to not over interpret data we decided to

do a nonparametric test at time points where ANOVA showed a difference and only accepted it as significant if the Wilcoxon test showed a $p < 0.05$.

Conclusion and Clinical relevance

With romifidine time to maximal effect is longer and action, after discontinuing CRI, prolonged compared to xylazine. The prolonged time to maximal effect should be taken into account under clinical situation in order to not stimulate the horses before sufficiently sedated.

The degree of sedation, based on HHAG, was the same with both α_2 -adrenergic agonists from 20 minutes after bolus administration to the end of CRI. However, based on reaction to acoustic stimulation by metallic noise, the horses might be more arousable with xylazine compared to romifidine.

Sedation with α_2 -adrenergic agonists decreases the reaction to visual, acoustic and tactile stimulation. However, response is not completely abolished and intensity might be dependent on the individual horse.

3.4. Trial 4: Effects on cardiopulmonary function and oxygen delivery of romifidine and xylazine doses followed by constant rate infusions (CRI) in standing horses

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3.4.1. Summary

Objective: The objective of this study was to compare the cardiopulmonary effects of a xylazine or romifidine loading-dose, followed by a constant rate infusion (CRI) of the same α_2 -agonist.

Materials and Methods: Nine research horses were treated in a randomized, blinded, crossover design with xylazine or romifidine. After instrumentation, an intravenous xylazine (1 mg kg^{-1}) or romifidine ($80 \text{ } \mu\text{g kg}^{-1}$) loading dose was administered, immediately followed by a CRI of xylazine ($0.69 \text{ mg kg}^{-1}\text{hour}^{-1}$) or romifidine ($30 \text{ } \mu\text{g kg}^{-1}\text{hour}^{-1}$) for a duration of 2 h. Cardiopulmonary variables were recorded before bolus administration, during CRI, and for 1 h after discontinuing drug administration.

Results: A significant decrease in haemoglobin concentration (tHb), arterial oxygen content (CaO_2), oxygen delivery (DO_2), mixed venous partial pressure of oxygen, heart rate, and cardiac output (Qt) followed the loading dose with both treatments. Carotid arterial blood pressure (ABP), systemic vascular resistance, and right atrial pressure (RAP) increased significantly. The increased ABP was followed by a significant decrease compared to baseline. Mean pulmonary arterial pressure increased significantly with romifidine only. No significant changes in stroke volume, arterial partial pressure of oxygen, and oxygen consumption were observed. Changes in Qt and RAP were more pronounced with romifidine. During CRI, tHb, and CaO_2 were significantly higher with romifidine, whereas DO_2 did not differ between treatments.

Conclusion and Clinical Relevance: cardiopulmonary effects were more pronounced and lasted longer with romifidine compared to xylazine. However, during CRI DO_2 was not different between drugs. With both α_2 -agonists, cardiovascular effects were most pronounced after loading dose administration and tended to stabilize during CRI.

3.4.2. Introduction

Alpha₂-adrenergic agonists are the most frequently used sedatives in equine medicine, and the only ones providing reliable sedation for standing surgical procedures. However, alpha₂-adrenergic agonists produce serious adverse effects on the cardiovascular system of horses (Yamashita et al. 2000; Freeman et al. 2002; Bettschart-Wolfensberger et al. 2005).

For prolonged standing sedation, frequent re-dosing of alpha₂-adrenergic agonists is necessary. Therefore, the use as a constant rate infusion (CRI) is becoming increasingly popular. By using CRI, constant plasma concentrations and therefore constant sedation are achieved. Additionally, Bettschart-Wolfensberger et al. (1999b) described that the cardiovascular effects observed after a loading dose of medetomidine stabilize during subsequent CRI whilst sedation is maintained. Detomidine (Daunt et al. 1993; Wilson et al. 2002; van Dijk et al. 2003; Aguiar et al. 2009), xylazine (Ringer et al. 2012a), medetomidine (Bettschart-Wolfensberger et al. 1999a, 1999b; Solano et al. 2009), and romifidine (Ringer et al. 2012b) have been described as CRIs for prolonged sedation of horses. However to date, cardiovascular effects have only been studied in detail for medetomidine and detomidine CRI (Daunt et al. 1993; Bettschart-Wolfensberger et al. 1999b; Aguiar et al. 2009). Whilst detomidine, romifidine and xylazine are licensed in horses, medetomidine is not. Compared to equi-sedative doses of detomidine, romifidine produces less ataxia (England et al. 1992; Hamm et al. 1995) and xylazine exerts less effect on heart rate (England et al. 1992).

Despite the documented advantages of xylazine and romifidine over other alpha₂-adrenergic agonists, the cardiopulmonary effects of romifidine and xylazine CRIs have not been studied for standing sedation. Additionally, to the authors' knowledge, the cardiopulmonary effects of two different alpha₂-adrenergic agonists CRIs have never been compared in horses.

Therefore, the goal of the present study was to investigate and compare the cardiopulmonary effects of romifidine or xylazine loading doses followed by CRI of the same drug in horses.

3.4.3. Materials and Methods

This study was approved by the Ethical Committee of the National Veterinary School of Lyon (N°0807, May 13th 2008).

Nine healthy adult research horses (4 geldings, 5 mares; 8 French Standardbreds, 1 French Saddlebred) with an age of 8.3 ± 1.8 years (mean \pm SD) (range 5.7 – 11.8 years) and a body weight of 517 ± 49 kg (range 430 – 580 kg) were included in the study.

Before experimentation, animals were acclimatised over several weeks and trained to enter and stand quietly in a stock. One carotid artery from each horse was surgically translocated to a subcutaneous position at least three months before the experiment.

After clinical examination, horses were placed in a stock and equipped with a base-apex electrocardiogram (ECG) (Datex-Ohmeda AS3 Anandic Medical Systems, Diessenhofen, Switzerland).

Catheters were placed after local infiltration of lidocaine (2 ml per injection site) (Xylovet, CEVA Santé Animale, Libourne, France). An arterial catheter 18G x 23 cm (Arterial Catheterization Set 18G x 23 cm, Arrow Swiss GmbH, Baar, Switzerland) was introduced into the raised carotid artery. Two 8.5F introducer sheaths (Intro-flex, Edwards Lifesciences, Horw, Switzerland) were placed into the contralateral jugular vein. Through the caudal introducer sheath, a 7F 110 cm Swan Ganz catheter (Thermocath Plus 4 lumen 110 cm 7F, Vygon, Aachen, Germany) was advanced into the pulmonary artery. An 8F 110 cm angiography catheter (Berman Angiographic Ballon Catheter: 110 cm, Arrow Swiss GmbH, Baar, Switzerland) was advanced into the right ventricle via the cranial sheath and then retracted for final positioning into the right atrium. Catheter placement was performed under pressure-tracing guidance. Systemic arterial blood pressure (ABP), right atrial pressure (RAP), mean pulmonary arterial blood pressure (MPAP), and pulmonary capillary wedge pressure (PCWP) were measured by connecting the respective catheter ports via fluid-filled lines to calibrated pressure transducers (Pressure Transducer DTX/Plus; Becton Dickinson, Allschwil, Switzerland). The transducers were zeroed to ambient pressure and all were positioned at the level of the scapulo-humeral joint for measurements.

Cardiac output (Qt) was measured using the thermodilution technique. A bolus of 30 mL ice-cold 5% glucose solution (Glucose 5% B. Braun, B. Braun Medical, Boulogne, France) was manually injected over approximately 2 seconds into the right atrium. An inline temperature sensor (BD CO In-Line Sensor, Becton-Dickinson, Allschwil, Switzerland) was connected to the angiography catheter for temperature measurement during injection. The curves obtained were evaluated visually and measurements repeated until three reliable curves based on shape were obtained. A maximum of 5 consecutive measurements per time point were performed. The mean of the curves was used for statistical analyses.

Blood pressures, ECG, blood temperature, and Qt were measured and displayed using a multiparameter monitor (Datex-Ohmeda AS3 Anandic Medical Systems, Diessenhofen, Switzerland). For subsequent analysis, all data were recorded in raw-data format on a personal computer using specific software (Datex-Ohmeda S/5TM Collect, GE Healthcare,

Diessenhofen, Switzerland). At predetermined time points, a mixed venous and an arterial blood sample were anaerobically collected from the pulmonary and carotid artery, respectively. Samples were collected into blood gas syringes (BD A-Line™, BD Diagnostics, Plymouth, UK) and immediately analyzed using a point-of-care blood gas system including a co-oximeter (Rapidpoint® 400, Siemens Medical Solutions Diagnostics, Saint Vulbas, France).

After instrumentation, two baseline measurements (B1, B2) of all variables were taken 10 minutes apart. The order of data collection was standardized as follows: HR, ABP, RAP, MPAP, PCWP, Qt and collection of blood samples.

The study was performed using a blinded crossover design. Horses were randomly allocated to receive xylazine or romifidine first, with a 16-day washout period between treatments. After baseline measurements, horses were sedated with xylazine (Xylasol ad us. vet., Dr. E. Graeub, Bern, Switzerland) (1 mg kg^{-1}) or romifidine (Sedivet®, Boehringer Ingelheim, Paris, France) ($80 \text{ } \mu\text{g kg}^{-1}$) given intravenously (IV) over 3 minutes by manual injection through the side arm of one of the introducer sheaths positioned in the jugular vein. The start of the loading dose administration was time point 0 and was immediately followed by xylazine ($0.69 \text{ mg kg}^{-1}\text{hour}^{-1}$) or romifidine ($30 \text{ } \mu\text{g kg}^{-1}\text{hour}^{-1}$) for 2 hours (time points 3 – 123 min). The CRIs were delivered by an infusion pump (Syramed®µSP6000, Arcomed, Regensdorf, Switzerland). Drugs were diluted in 0.9% saline (Chlorure de Sodium 0.9% B. Braun; B. Braun Medical, Boulogne Cedex, France) by a third person so that equal volumes were administered in both treatments.

During loading dose administration, HR, mean arterial blood pressure (MAP), MPAP, and RAP were recorded after one and two minutes. Thereafter all parameters (HR, MAP, MPAP, RAP, PCWP, Qt and blood gases) were recorded every 10 minutes during the first hour of CRI (13, 23, 33, 43, 53, 63 min) and every 15 minutes during the second hour (78, 93, 108, 123 min). Constant rate infusions were discontinued after 2 hours (time point 123 min) and data were collected every 15 minutes for an additional hour (138, 153, 168, and 183 min).

In order to avoid errors due to artefacts, all blood pressure curves and HR were re-analyzed based on the raw-data recordings at the end of the experiment using specific software (Datex-Ohmeda S/5™ Collect, GE Healthcare, Diessenhofen, Switzerland). At time points 1 and 2 mean values from the first 15 seconds, starting exactly at the measurement point, were recorded. Afterwards mean values of the first 30 seconds of the different time-points were used. Time frames with artefacts were skipped to avoid bias in results.

Cardiac index [CI (mL min⁻¹kg⁻¹) = Qt/bwt], stroke volume [SV (L/beat) = Qt/HR], systemic vascular resistance [SVR (dyn s cm⁻⁵) = [(MAP – RAP)/Qt × 80]], oxygen content in mixed venous [CvO₂ (mL O₂/dL blood) = (1.39 × Hb (g dL⁻¹) × SvO₂ (%/100)) + 0.003 × PvO₂] and arterial blood [CaO₂ (mL O₂/dL blood) = (1.39 × Hb (g dL⁻¹) × SaO₂ (%/100)) + 0.003 × PaO₂], oxygen delivery [DO₂ (mL O₂/min) = Qt × CaO₂ × 10], oxygen consumption [VO₂ (mL O₂/min) = Qt × (CaO₂ – CvO₂) × 10] and oxygen extraction ratio [ERO₂ = [(CaO₂ – CvO₂) / CaO₂] × 100(%)] were calculated.

Statistical analysis was performed using SigmaStat[®] 3.5 (SigmaStat[®] 3.5, Systat Software GmbH, Erkrath, Germany). Two-way repeated measures ANOVA (two within factors: treatment and time) followed by a Holm-Sidak test for multiple comparisons versus a control were used to compare the two treatments and to study changes over time compared to baseline. The time point B1 was considered the control. Validity of the normality assumption was confirmed by assessment of normal probability plots of the residuals. The level of significance was set at $p < 0.05$.

3.4.4. Results

All data are presented as mean ± SD. For all variables, there were no significant differences between baseline values of the two treatments and between the two baseline measurements (B1, B2) within treatment.

Cardiovascular variables are shown in Fig. 11.

For both treatments, an initial decrease in HR, Qt and CI, an increase in SVR and RAP, and an increase followed by a significant decrease in MAP were observed following the loading dose. Mean pulmonary arterial pressure increased with romifidine but not with xylazine treatment. No significant changes in SV were observed.

The initial changes in HR, MAP, RAP, and SVR persisted longer with romifidine compared to xylazine during the CRI. Effects on Qt and RAP were more profound with romifidine.

Due to the length of the catheters and the size of the horses, PCWP could not be monitored continuously and data was not used for statistical analysis due to large number of missing values. Analysis of MPAP was only performed for 5 horses due to missing values.

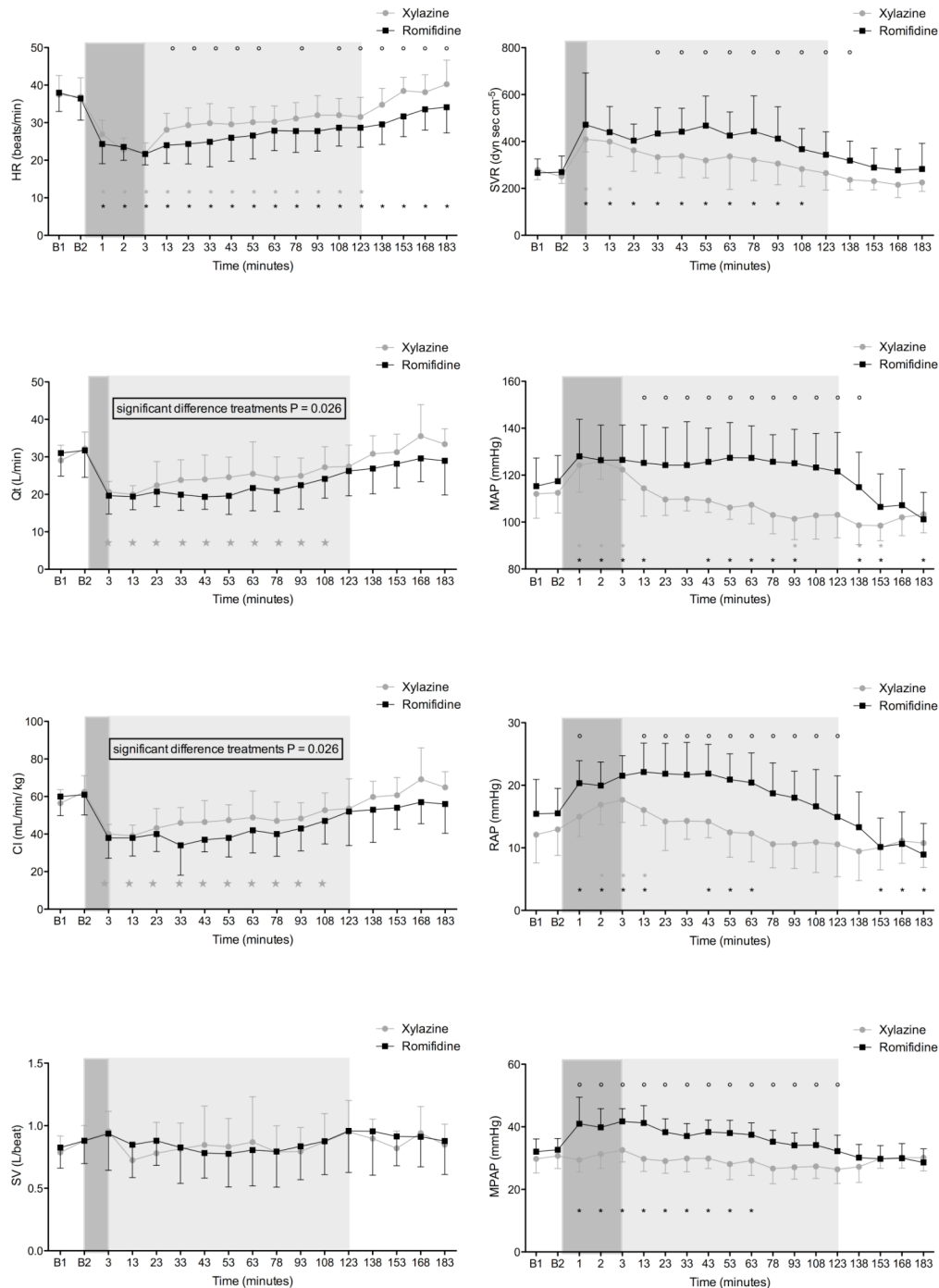


Figure 11: Cardiovascular variables (mean \pm SD) of nine research horses before (time points B1, B2) and during drug administration (loading dose of xylazine 1 mg kg^{-1} or romifidine $80 \text{ } \mu\text{g kg}^{-1}$ IV, time points 0 – 3 min; followed by a constant rate infusion of xylazine $0.69 \text{ mg kg}^{-1} \text{ hour}^{-1}$ or romifidine $30 \text{ } \mu\text{g kg}^{-1} \text{ hour}^{-1}$ for 2 hours, time points 3 – 123 min) and during an additional hour after stopping the α_2 -adrenergic agonists (time points 123 – 183 min).

* Significant difference ($P < 0.05$) compared to the baseline (B1) of the same treatment

° Significant difference ($P < 0.05$) between treatments

★ With both treatments significant difference compared to baseline (B1)

HR: heart rate, Qt: cardiac output, CI: cardiac index, SV: stroke volume, SVR: systemic vascular resistance, MAP: mean arterial blood pressure, RAP: right atrial pressure, MPAP: mean pulmonary arterial pressure.

Blood gas variables are presented in Fig. 12.

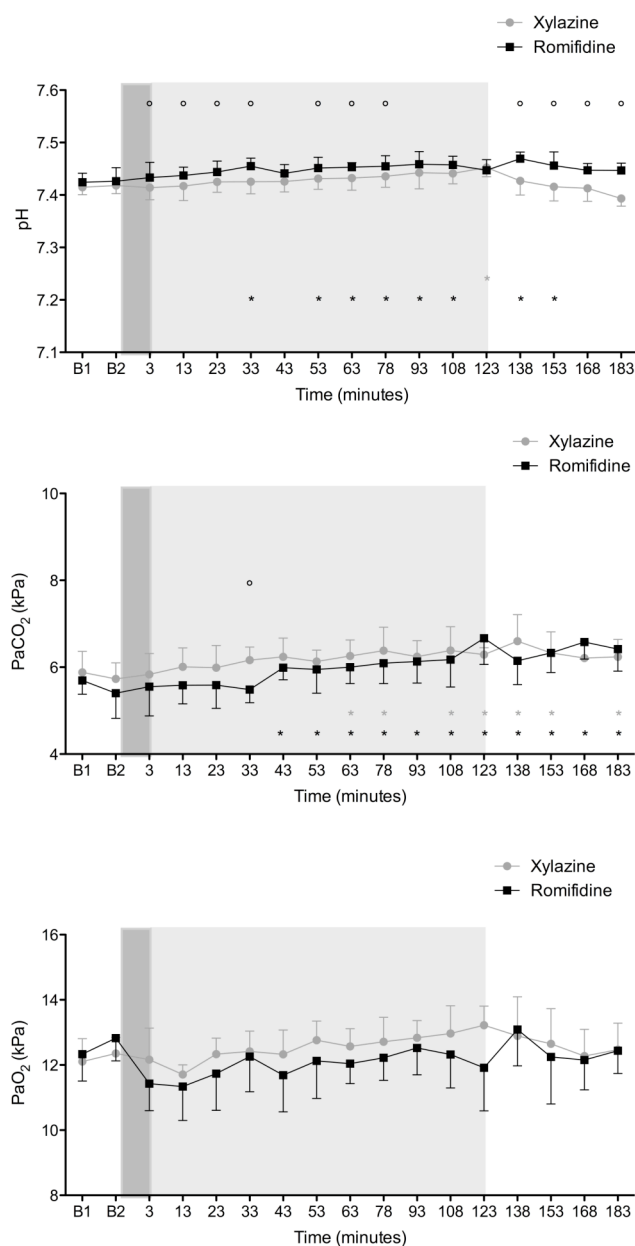


Figure 12: Course of the pH, arterial partial pressure of carbon dioxide (PaCO₂), and arterial partial pressure of oxygen (PaO₂) (mean \pm SD) of nine research horses before (time points B1, B2) and during drug administration (loading dose of xylazine 1 mg kg⁻¹ or romifidine 80 μ g kg⁻¹ IV, time points 0 – 3 min; followed by a constant rate infusion of xylazine 0.69 mg kg⁻¹hour⁻¹ or romifidine 30 μ g kg⁻¹hour⁻¹ for 2 hours, time points 3 – 123 min) and during an additional hour after stopping the α_2 -adrenergic agonists (time points 123 – 183 min).

* Significant difference ($P < 0.05$) compared to the baseline (B1) of the same treatment

° Significant difference ($P < 0.05$) between treatments

Data from one horse was excluded from statistical analysis due to technical problems with the blood gas analyser. There were no significant changes in arterial partial pressure of oxygen (PaO_2). Arterial pH and partial pressure of carbon dioxide (PaCO_2) increased significantly during CRI's administration with both xylazine and romifidine. The pH was slightly higher with romifidine compared to xylazine at 11 out of 17 measurement points.

With both drugs there was a significant decrease in total haemoglobin (tHb), arterial oxygen content (CaO_2), partial pressure of oxygen in mixed venous blood (PvO_2), and oxygen delivery (DO_2) as well as an initial increase in oxygen extraction ratio (ERO_2) immediately after bolus administration (Fig. 13). With romifidine, the decrease in tHb and CaO_2 occurred later.

Snoring and forced respiration was observed in all the horses. Subjectively, frequent urination was observed with both treatments, but frequency and volume of urine were not quantified.

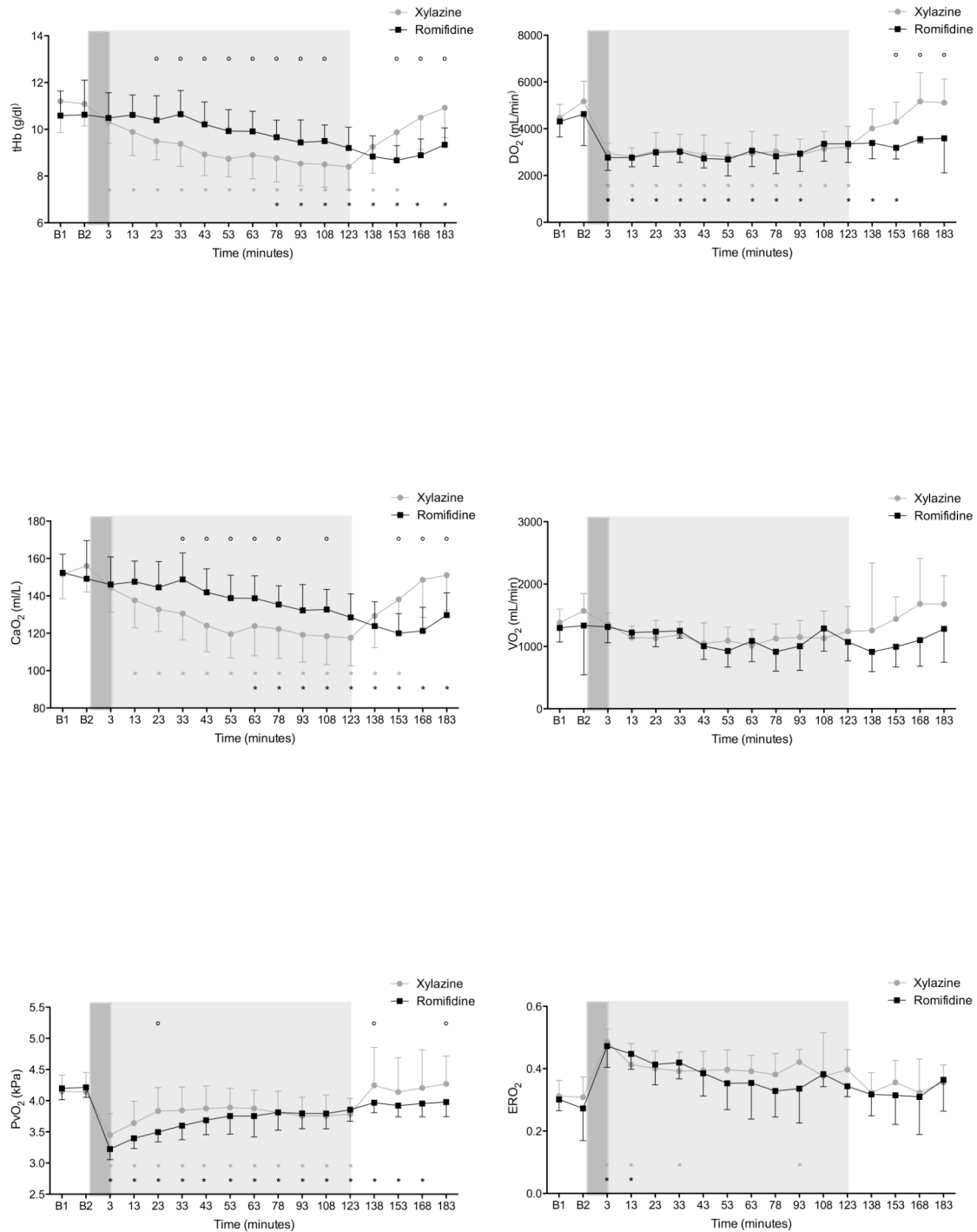


Figure 13: Variables related to oxygen delivery (DO₂) and consumption (VO₂) (mean \pm SD) of nine research horses. Measurements were recorded before (time points B1, B2) and during drug administration (loading dose of xylazine 1 mg kg⁻¹ or romifidine 80 μ g kg⁻¹ IV, time points 0 – 3 min; followed by a constant rate infusion of xylazine 0.69 mg kg⁻¹ hour⁻¹ or romifidine 30 μ g kg⁻¹ hour⁻¹ for 2 hours, time points 3 – 123 min) and during an additional hour after stopping the α_2 -adrenergic agonists (time points 123 – 183 min).

* Significant difference ($P < 0.05$) compared to the baseline (B1) of the same treatment

° Significant difference ($P < 0.05$) between treatments

tHb: total haemoglobin, CaO₂: arterial oxygen content, PvO₂: partial pressure of oxygen in mixed venous blood, ERO₂: oxygen extraction ratio.

3.4.5. Discussion

Typical cardiovascular changes for α_2 -adrenergic agonists in horses (England & Clarke 1996) were observed during xylazine and romifidine administration. Changes in RAP, Qt, and MPAP were more pronounced with romifidine, however DO_2 was not different compared to xylazine during drug administration. Overall, cardiovascular effects lasted longer with romifidine.

The loading doses used in the present study have been described as being equipotent (England et al. 1992), as well as the CRI doses to produce constant plasma concentrations and sedation (Ringer et al. 2012a, 2012b).

With both α_2 -adrenergic agonists a significant decrease in HR was observed after only one third of the loading dose. The HR was lowest during loading dose administration and increased afterwards during the CRI. However with both drugs, HR remained significantly decreased compared to baseline until discontinuing CRIs. The more prolonged decrease with romifidine was probably related to a longer depression of CNS sympathetic outflow and increase in peripheral vagal tone. Contrary to results reported by England et al. (1992), maximal changes in HR were not different between the two drugs.

A significant increase in MAP and SVR was observed during loading dose administration. Unlike romifidine, MAP with xylazine returned to baseline values immediately after loading dose administration. However, during the maximum increase there was no significant difference between treatments regarding MAP or SVR. The initial hypertension due to α_2 -adrenergic agonists is attributed to a peripherally mediated vasoconstriction and is followed by a prolonged centrally mediated hypotension (Wagner et al. 1991; Yamashita et al. 2000; Freeman et al. 2002). The observed transient increase in SVR (Fig. 11) reflecting vasoconstriction coincides with the increase in MAP. However, the exact mechanism leading to a decrease in MAP (both treatments) and RAP (romifidine only) in the face of normalized Qt and SVR at the end of the observation period is unknown. A possible cause might be a slight decrease in total circulating volume induced by diuresis. Unfortunately, urination was not quantified in the present study. However, in a study using the same horses and treatments (two hours of CRI), the authors observed that the mean number of episodes of urination was 2.1 and 3.5 for xylazine and romifidine, respectively. Horses did urinate significantly more frequently with romifidine compared to xylazine (Ringer et al. unpublished data).

Changes in RAP for romifidine were significantly higher and more prolonged compared to xylazine. The increase in RAP is probably caused by a combination of decreased HR and Qt, increased MPAP, and constriction of venous capacitance vessels. With romifidine only, a significant decrease in RAP compared to baseline was observed after stopping CRI (Fig. 11).

Cardiac output significantly decreased after loading dose administration, but returned back to baseline values already during the CRIs. Cardiac output was significantly lower with romifidine. A decrease in Qt can generally be attributed to a decrease in HR or a decrease in SV. A decrease in HR after xylazine and romifidine has been confirmed repeatedly, however the effects on SV are controversial (Wagner et al. 1991; Yamashita et al. 2000; Freeman et al. 2002). Stroke volume is determined by preload (ie, end-diastolic ventricular volume), afterload (ie, ventricular wall stress during ejection), and contractility (ie, velocity of actin-myosin crossbridge cycling at zero load). With the techniques used in the present study, these variables could not be directly measured. Nevertheless, decreased preload was considered unlikely because RAP increased simultaneously with the drop in Qt. Based on changes in MAP and SVR, changes in afterload may have been present. Although drug effects might have altered ventricular loading and myocardial contractility, no significant changes in SV were observed. Therefore, from the data obtained in the present study we inferred that the decrease in Qt was primarily caused by drug-induced bradycardia.

Significant changes in MPAP were seen only with romifidine. An increase in MPAP has been shown with detomidine, dexmedetomidine and romifidine in horses (Freeman et al. 2002; Bettschart-Wolfensberger et al. 2005; Nyman et al. 2009). The magnitude of the effect, as well as the duration is probably dose and drug dependent (Wagner et al. 1991; Ebert et al. 2000). The increase in MPAP with romifidine was already significant after only one third of the loading dose. The clinical importance of a transient increase MPAP in healthy, standing horses is probably negligible. However, when changes in vessel tone are desirable (eg, for regulation of ventilation-perfusion matching during periods of alveolar hypoxia) or in horses presenting pulmonary or cardiovascular diseases, the altered vessel tone might become important. Due to unreliable recording of PCWP, pulmonary vascular resistance could not be calculated. However, based on results of other studies with α_2 -adrenergic agonists, it is likely that the increase in MPAP was caused by pulmonary vasoconstriction (Wagner et al. 1991; Nyman et al. 2009).

Previous investigations reported no significant decrease in PaO₂ after single bolus doses of xylazine and romifidine (Muir et al. 1979a; Yamashita et al. 2000; Freeman et al. 2002) in agreement with our results. Conversely, other authors reported a decrease in PaO₂ at the same or even lower bolus doses than we used as a loading dose (Gasthuys et al. 1990; Clarke et al. 1991; Lavoie et al. 1992).

A significant reduction in haematocrit has been reported after xylazine and detomidine administration (Gasthuys et al. 1990; Wagner et al. 1991; Daunt et al. 1993). In the present study, a decrease in tHb was observed with both xylazine and romifidine (Fig. 13). However, with xylazine, the decrease was much earlier. As there was no significant decrease in PaO₂ with either drug, the decrease in tHb was most likely the reason for the decrease in CaO₂.

Studies on the cardiovascular effects of alpha₂-adrenergic agonists rarely investigate the effects on DO₂. Oxygen delivery is dependent on Qt and CaO₂. Despite a lower Qt with romifidine, there was no significant difference in DO₂ between treatments up to the end of drug administration. This can be attributed to the higher tHb with romifidine from 23 to 108 min. Interestingly, DO₂ increased immediately after discontinuing xylazine but not romifidine, coinciding with the immediate and significant increase in tHb concentrations and the normal Qt after stopping xylazine.

The exact mechanism leading to a decrease in tHb with alpha₂-adrenergic agonists is unknown. A fluid shift from the extra to intravascular compartment to maintain Qt (Wagner et al. 1991), as well as pooling of erythrocytes in the spleen or other reservoirs due to reduced sympathetic tone (Gasthuys et al. 1990; Wagner et al. 1991) have been suggested as possible causes. The shift of fluids into the intravascular space because of low Qt would not explain the higher tHb measured with romifidine, since romifidine treatment caused a lower Qt. Therefore pooling of erythrocytes in the spleen or other reservoirs due to a vasodilatation seems to be more likely. With romifidine, the initial vasoconstriction is prolonged compared to xylazine, which could explain the later onset of the reduction in tHb due to vasodilation. In agreement with this theory, Daunt et al. 1993 showed that a higher SVR (related to a higher dose of detomidine) was not accompanied by a decrease in haematocrit, whereas a lower SVR (due to a lower dose of detomidine) was accompanied by a decrease in haematocrit.

A decrease in PvO_2 has been described after α_2 -adrenergic agonists administration (Freeman et al. 2002; Nyman et al. 2009). The fall in PvO_2 is related to an increased ERO_2 , resulting from a decreased DO_2 in face of an unchanged VO_2 (Fig. 13). Contrary to our results, a decrease in VO_2 after administration of α_2 -adrenergic agonists has been reported in other studies (Daunt et al. 1993; Taittonen et al. 1997). The effects of α_2 -agonists on DO_2 and VO_2 in the peripheral tissues remain to be investigated.

With both xylazine and romifidine, a significant increase in $PaCO_2$ during CRI administration was detected. Despite the $PaCO_2$, a significant increase in pH was observed with both drugs. An increase in BE was observed during a medetomidine CRI (Bettschart-Wolfensberger et al. 1999b), and is probably the cause of changes in pH in the present study.

Snoring and forced respiration due to upper airway obstruction was observed in all horses. Forced inspiration and expiration affects intrathoracic pressures and therefore influences cardiovascular measurements. In order to avoid errors due to measurements during different phases of the respiratory cycle, mean values averaged over 30 s of the different variables were used at each time point, including both inspiratory and expiratory phases.

Compared to studies reporting cardiovascular effects after single bolus doses of α_2 -agonists, cardiovascular effects seem to be prolonged if a CRI is started after loading dose administration (Clarke et al. 1991; Wagner et al. 1991; Yamashita et al. 2000; Freeman et al. 2002). This could be attributed to persistently higher plasma concentrations over time when a CRI is added compared to single bolus doses.

Maximal cardiovascular changes occur during or immediately after loading dose administration and most of the cardiovascular variables stabilize during CRI. In agreement with Bettschart et al. (1999b), the administration of a single loading dose followed by CRI will likely be advantageous over repeated boli administration. However, to the authors knowledge there is no study investigating the cardiovascular effects during repeated boli of xylazine or romifidine in horses.

The prolonged duration of cardiopulmonary effects with romifidine compared to xylazine is not surprising, as a prolonged sedative effect of romifidine has already been described (England et al. 1992; Nannarone et al. 2007). The prolonged effect of romifidine might be due

to a reservoir in the corpuscular fraction of blood leading to a slow release into the circulation (Romagnoli et al. 2009). The greater effect of romifidine on some (Qt, RAP and MPAP) but not all variables might be explained by the higher α_2/α_1 selectivity ratio of romifidine. Another possible explanation would be a difference in selectivity to the different α_2 -adrenergic receptor subtypes (α_{2A} , α_{2B} , α_{2C}) between xylazine and romifidine. The three α_2 -adrenergic receptor subtypes can be differently distributed among vessels of the different body areas and also differently involved on vessel activity depending on the localization (Gornemann et al. 2007).

Conclusions

In general, cardiovascular depression was more severe and prolonged with romifidine. However, no differences in DO_2 could be detected during drug administration. Most of the cardiovascular effects were most pronounced during loading dose administration and tended to stabilize during CRI. Therefore, CRI is likely to be safer than repeated boli administration for prolonged sedation of horses using α_2 -adrenergic agonists. Xylazine might be safer than romifidine in cardiovascularly compromised horses.

4. Overall discussion and outlook

4.1. Overall discussion

Constant rate infusion protocols providing stable sedation with constant plasma concentrations were successfully developed for xylazine and romifidine. Adding butorphanol did not reduce the α_2 -adrenergic agonist requirements. Additionally, dangerous postural instability as well as inconsistent sedation was observed when butorphanol was combined with xylazine. Equal degree of sedation judged by head lowering was achieved with both α_2 -adrenergic agonists. However with romifidine the horses were less arousable by acoustic stimulation and there was a tendency to less ataxia compared to xylazine. With romifidine some cardiovascular effects were more pronounced. However, oxygen delivery was not different to xylazine during constant rate infusion. After discontinuing drug administration, sedation and effects on cardiopulmonary function were more prolonged with romifidine compared to xylazine.

4.1.1 Development of sedation protocols by observing lowering of the head

The development of the sedation protocols (trial 1 and 2) was based on observation of the head height above the ground (HHAG). Similar materials and methods were successfully used before to develop a medetomidine CRI protocol providing stable sedation and plasma concentrations in horses (Bettschart-Wolfensberger et al. 1999a). Kollias-Baker et al. (1993) used a xylazine CRI of $12 \mu\text{g kg}^{-1}\text{minute}^{-1}$ ($0.72 \text{ mg kg}^{-1}\text{hour}^{-1}$) to achieve plasma concentrations between $400 - 800 \text{ ng mL}^{-1}$ and moderate sedation (Kollias-Baker et al. 1993). The dose rate of xylazine was calculated based on experimental kinetic data and the resulting dose rate was similar to dose rates determined in our study during part I of trial 1. However, this was the first time the method was used with a longer acting α_2 -adrenoceptor agonist like romifidine (England et al. 1992; England & Clarke 1996). No accumulation, defined as increasing plasma concentrations with time of infusion, was observed with romifidine after two hours of CRI. But rather, compared to data after single bolus administration (England et al. 1992; Hamm et al. 1995), recovery in our study seemed to be even faster after discontinuing the CRI compared to after romifidine bolus administration.

The measurement of the distance between head and ground (lowering of the head) has been frequently used to objectively assess degree of sedation provided by α_2 -adrenergic agonists (Bryant et al. 1991; Clarke et al. 1991; England et al. 1992; Hamm et al. 1995; Freeman & England 1999; Freeman & England 2000; Figueiredo et al. 2005; Nannarone et al.

2007). Advantages of the HHAG are that it is a quantitative and objective variable, and that the horses do not need to be stimulated for measurement. However, it might be difficult to differentiate degree of sedation from quality of sedation in unstimulated horses. Actually, HHAG might be more indicative for degree than for quality of sedation (Hamm et al. 1995). Therefore in trial 3 we included different parameters in addition to the HHAG to further assess quality of sedation.

4.1.2 The addition of butorphanol to alpha₂-adrenergic agonist constant rate infusions

Based on the frequent combination of alpha₂-adrenergic agonists with opioids in equine practice, the addition of butorphanol to the developed CRIs was studied. Although there was an initial effect of the loading dose of butorphanol on time to first additional romifidine requirements, over all, butorphanol did not reduce xylazine or romifidine requirements. Contrary to our results, a synergistic effect regarding sedation (Corletto et al. 2005; DeRossi et al. 2009), analgesia (Robertson & Muir 1983; Schatzman et al. 2001; Kohler et al. 2004) and response to stimulation (Paton & Clarke 1986; Clarke & Paton 1988; Clarke et al. 1991; Holopherne et al. 2005) has been described by adding opioids to alpha₂-adrenergic agonists. Based on these results, in clinical situations alpha₂-adrenergic agonists are often combined with opioid drugs in order to improve effectiveness of sedation. Aguiar et al. 2009 reported similar sedation scores when a CRI of detomidine was used in combination with butorphanol compared to a higher dose detomidine CRI without butorphanol. However, in agreement with our results, other authors did not observe more profound sedation when butorphanol was administered with detomidine or romifidine (Clarke et al. 1991; Love et al. 2011). The methods used in the present study have been used the first time to test the effects of additional butorphanol to alpha₂-adrenergic agonists sedation. As with the HHAG degree of sedation rather than quality of sedation are tested, the study design may not have been ideal for this purpose. The horses were not stimulated and were pain free, therefore the positive effect that butorphanol may have in clinical cases, could have been missed. Nevertheless, the longer time until the first additional dose of romifidine observed during part I of trial 1, the higher ataxia scores observed with romifidine-butorphanol during part II, as well as the initially increased ataxia after adding butorphanol to xylazine may be indicative of deeper sedation with butorphanol.

On the other hand, despite similar doses of alpha₂-adrenergic agonist, inconsistent sedation was observed when butorphanol was added to the xylazine protocol. Theoretically this could

be due to an excitatory effect of butorphanol not abolished completely by xylazine. This is rather unlikely, as at the butorphanol doses used in the present study no adverse behavioural effects were observed in healthy horses (Sellon et al. 2001), although no concomitant α_2 -adrenergic agonist was administered. Xylazine may have an effect on metabolism and clearance of butorphanol leading to plasma concentrations producing an excitatory effect. Unfortunately plasma concentrations of butorphanol were not measured in the present trials. No inconsistent sedation was observed when the same doses of butorphanol were added to romifidine. This might be due to a deeper sedation with romifidine compared to xylazine. Even though this could not be confirmed in trial 3 where the two α_2 -adrenergic agonists were directly compared for degree of sedation based on HHAG. However, as mentioned earlier head height is only one parameter to evaluate sedation and therefore during trial 3 the horses were subjected to acoustic, visual and tactile stimulation while sedated with a xylazine or romifidine CRI. An increased reaction to metallic noise stimulation with xylazine was observed despite no differences in HHAG compared to romifidine. This means, that even with no difference in HHAG, quality of sedation and thus arousability might be different. A reduced quality of sedation with xylazine could be an explanation for higher arousability with this drug compared to romifidine leading to insufficient sedation when a light excitatory effect, as caused by butorphanol, is present.

Ataxia is an important effect of α_2 -adrenergic agonists. Horses unsteadiness is uncomfortable for surgeons and might become dangerous to horse and surgeon if the horse falls down. It was surprising that some horses fell down when butorphanol was added to xylazine, as the dose rates used were within commonly recommended dose rates. Some authors even described higher dose rates of butorphanol in combination with α_2 -adrenergic agonist in experimental studies (Robertson & Muir 1983; Brunson & Majors 1987; Rutkowski et al. 1991). Unacceptable degree of ataxia and/or falling down after α_2 -adrenergic agonist treatment (Freeman & England 2000) or after combination of high doses of α_2 -adrenergic agonist with opioids (Paton & Clarke 1986; Clarke & Paton 1988; Greene & Thurmon 1988) has been observed previously. Paton & Clarke (1986) observed that severe ataxia might be a serious side effect if an opiate was added to horses already ataxic because of an α_2 -adrenergic agonist. With romifidine, ataxia was considered acceptable with and without butorphanol, however horses were significantly more ataxic when butorphanol was added.

Based on our results (trial 1), the tested xylazine-butorphanol protocol cannot be recommended. Due to the inconsistent sedation and the dangerous postural instability observed when butorphanol was added to the xylazine protocol (trial 1), and because no α_2 -adrenergic agonist sparing effects were observed with butorphanol with either α_2 -adrenergic agonist (trial 1 and 2), trials 3 and 4 were performed without butorphanol.

4.1.3 Comparison of the xylazine vs romifidine constant rate infusion protocol regarding degree and quality of sedation

This was the first time two α_2 -adrenergic agonist CRIs were compared experimentally regarding degree and quality of sedation.

During CRI degree of sedation based on HHAG was not different between the two α_2 -adrenoreceptor agonists. In general, sedation with α_2 -adrenergic agonists decreased the reaction to tactile, acoustic and visual stimulation compared to baseline. Besides reaction to metallic noise there was no significant difference between the two α_2 -adrenoreceptor agonists regarding reaction to different type of stimulation. With romifidine, reaction to metallic noise was significantly less and reaction to other type of stimuli was decreased for a longer time period after discontinuing CRI compared to xylazine.

Overall a decrease in reaction to touch was observed after α_2 -adrenoreceptor agonist administration. However, and coinciding with England & Clarke 1996, some horses showed intense reaction to touch despite sedation. The intensity of reaction was more dependent on the individual horse than on the α_2 -adrenoreceptor agonist used.

The decreased reaction to metallic noise despite no difference in HHAG might have been due to a better quality of sedation with romifidine or due to a deeper sedation despite equal HHAG. On the other hand, it is known that α_2 -adrenergic receptors are involved in the involuntary reaction to a sudden unexpected stimulus for example noise (Startle reflex) (Quaglia et al. 2011). Therefore the different selectivity of xylazine and romifidine to the different α_2 -adrenergic receptor subtypes might lead to different response to the different stimuli.

Quality and degree of sedation are very difficult to differentiate, and HHAG is probably also influenced by the muscle relaxant properties of the α_2 -adrenoreceptor agonist. To further elucidate differences in quality of sedation clinical trials comparing the two protocols are very important.

Previous studies showed that instability and ataxia were significantly less with romifidine compared to other α_2 -adrenergic agonists when single doses were applied (England et al. 1992; Hamm et al. 1995; Nannarone et al. 2007). This could not be confirmed with trial 3 where xylazine and romifidine were compared for postural instability and ataxia during motion. However, there was a tendency of increased ataxia with xylazine, which might have become significant with a higher number of horses.

4.1.4 Comparison of the xylazine vs romifidine constant rate infusion protocol regarding cardiopulmonary function

Typical cardiovascular changes for α_2 -adrenergic agonists in horses (England & Clarke 1996) were observed during loading dose followed by CRI of xylazine and romifidine, respectively. A significant decrease in heart rate (HR), cardiac output (Qt), and mixed venous partial pressure of oxygen (PvO₂) were observed after loading dose administration. Systemic vascular resistance (SVR), right atrial pressure (RAP), and arterial blood pressures (ABP) increased significantly. The increase in ABP with both α_2 -adrenergic agonists and the increase in RAP with romifidine were followed by a significant decrease compared to baseline. An increase in mean pulmonary arterial pressure (MPAP) was observed with romifidine only. No significant changes in stroke volume (SV) were observed. Arterial pH and partial pressure of carbon dioxide (PaCO₂) increased significantly during CRI administration with both α_2 -adrenergic agonists. pH was slightly higher with romifidine compared to xylazine treatment in 11 of the 17 time points. There were no significant changes in arterial partial pressure of oxygen (PaO₂). However, a significant decrease in total haemoglobin concentration (tHb), arterial oxygen content (CaO₂), oxygen delivery (DO₂), as well as an initial increase in oxygen extraction ratio (ERO₂) immediately after bolus administration was observed. Changes in RAP, Qt, and MPAP were more pronounced with romifidine, however DO₂ was not different compared to xylazine during drug administration.

Interesting is that maximal changes in HR, ABP, and SVR did not differ between the two α_2 -adrenergic agonists. This is in contrast to other studies where maximal effects were less with xylazine compared to other α_2 -adrenergic agonists (England et al. 1992; Yamashita et al. 2000). However, most of the cardiovascular studies investigating xylazine probably missed the maximal effects, as data analysis started only 5 minutes after drug administration. Based on our observations, important changes occur during the first few minutes and many of them already during the first minute of xylazine or romifidine

administration. For example, with xylazine and romifidine the initial increase in ABP is the same, however with xylazine ABP returns to baseline values immediately after loading dose administration. Therefore, when performing cardiovascular studies with α_2 -adrenergic agonists, important data will be missed if recording is not started at the same time as drug administration.

Also surprising was the higher tHb and therefore CaO_2 with romifidine during drug administration leading to similar DO_2 during drug administration with both α_2 -adrenergic agonists despite a significantly lower Qt with romifidine.

Studies on the cardiovascular effects of α_2 -adrenergic agonists rarely investigate the effects on DO_2 . Emphasis is frequently centred on PaO_2 and Qt, but tHb is not measured and DO_2 is not calculated. To our knowledge, comparison of DO_2 between different α_2 -adrenergic agonists has not yet been reported. Oxygen delivery is dependent on Qt and CaO_2 . Since both Qt and CaO_2 decreased with treatment in the present study, DO_2 also decreased. Despite a lower Qt with romifidine compared to xylazine, there was no significant difference in DO_2 between treatments up to the end of drug administration. This can be attributed to the higher tHb with romifidine from 23 to 108 min. Interestingly, DO_2 increased immediately after discontinuing CRI with xylazine but not with romifidine, coinciding with the immediate and significant increase in tHb concentrations and the normal Qt after discontinuation of xylazine treatment. Based on our data the decrease in tHb occurring in both groups could be attributed to pooling of erythrocytes in the spleen or other reservoirs due to vasodilatation. With romifidine, the initial vasoconstriction is prolonged compared to xylazine, which would explain the later onset of reduction in tHb due to vasodilation.

The fall in PvO_2 is related to an increased ERO_2 , resulting from a decreased DO_2 in face of an unchanged oxygen consumption (VO_2). Contrary to our results, a decrease in VO_2 after administration of α_2 -adrenergic agonists has been reported in other studies (Daunt et al. 1993; Taittonen et al. 1997).

The importance of the more severe changes with romifidine in Qt, MPAP and RAP in face of equal DO_2 compared to xylazine remains to be investigated in more detail. Also the effects of α_2 -agonists on DO_2 and VO_2 in the peripheral tissues need to be investigated.

The greater effect of romifidine on some (Qt, RAP and MPAP) but not all variables might be explained by the higher α_2/α_1 selectivity ratio of romifidine. Another possible explanation would be a difference in selectivity to the different α_2 -adrenergic receptor subtypes (α_{2A} , α_{2B} , α_{2C}) between xylazine and romifidine. The three α_2 -adrenergic receptor subtypes can be differently distributed among vessels of the different body areas and also differently involved on vessel activity depending on the localization (Gornemann et al. 2007).

Over all, and coinciding with Bettschart et al. (1999b), a tendency to stabilization of cardiovascular parameters was observed already during CRI. However, cardiovascular depression was longer with romifidine compared to xylazine.

4.1.5 Xylazine vs romifidine constant rate infusion protocol: time to onset and duration of effects

Based on HHAG, with romifidine time to maximal sedative effect is longer compared with xylazine. The sedative as well as the cardiovascular effects are prolonged after romifidine compared to xylazine. Some cardiovascular and sedative effects persisting even one hour after discontinuing romifidine CRI. The prolonged effect with romifidine compared to xylazine is not surprising, as a prolonged sedative effect of romifidine has already been described (England et al. 1992; Nannarone et al. 2007). With xylazine, detomidine and medetomidine it was shown that the duration of cardiovascular depression seems to parallel the duration of sedation (Yamashita et al. 2000). The prolonged effect of romifidine might be due to a reservoir in the corpuscular fraction of blood leading to a slow release into the circulation (Romagnoli et al. 2009).

4.1.6 Other effects observed during α_2 -adrenergic agonist constant rate infusions

Clarke et al. (1991) observed muzzle tremors and head shaking when butorphanol was added to romifidine. These observations were interpreted as adverse effects of butorphanol. During trial 2, there was a tendency of more frequent shaking/trembling of head/muzzles with romifidine-butorphanol compared to romifidine, which was not statistically significant. However, occasional shaking and trembling of the head or muzzle was observed with both treatments and can therefore not be attributed to the opioid only. The presentation of shaking/trembling of head or muzzles might also be related to depth of sedation rather than to opioid administration.

Snoring due to upper airway obstruction is frequently observed during prolonged sedation of horses using α_2 -adrenergic agonists (England & Clarke 1996) and is caused mainly by gravitational forces causing mucosal oedema when the head is below its normal position. Snoring and forced respiration was observed in all horses. In order to not disturb the horses, no efforts were made to reduce obstruction, and no complications were observed. However if the CRIs are used over a prolonged time period, horses should be monitored for upper airway obstruction. To reduce swelling of nasal cavities the head could be maintained in an elevated position.

Significant diuresis was observed in all the studies. Urination was significantly more frequent with romifidine during trial 3. An increased diuretic effect due to α_2 -adrenergic agonists has been reported before (England & Clarke 1996). The increased diuretic effect of romifidine compared to xylazine has never been reported. The increased frequency of urination with romifidine can be due to a more prolonged effect of romifidine or because of a more potent or different diuretic effect. A difference in diuretic response between xylazine and medetomidine has been reported in dogs (Talukder & Hikasa 2008). This difference was attributed to different selectivity and specificity to α_2 -adrenoceptors and/or imidazoline receptors. The difference on diuresis between xylazine and romifidine in horses could probably also be attributed to these differences. Different mechanisms leading to diuresis due to α_2 -adrenergic agonists have been discussed, however the exact mechanism remains unknown and is probably a result of multiple haemodynamic, neural, hormonal and local factors in the kidney (Talukder & Hikasa 2009). The importance of the diuretic effect of α_2 -adrenergic agonists on electrolytes and cardiovascular function is insufficiently studied. Future studies are needed to investigate the effects of increased diuresis, especially in already dehydrated/hypovolemic animals.

Sweating has been reported during α_2 -adrenergic agonist sedation (England & Clarke 1996). During repeated sedation of the same horses over the four trials, sweating occurred but seemed to be rather dependent on ambient temperature than on α_2 -adrenergic agonist used (personal opinion).

4.2. Conclusion

With romifidine time to maximal effect is longer and action, after discontinuing CRI, prolonged compared to xylazine. The prolonged time to maximal effect should be taken into account under clinical situations in order to not stimulate the horses before maximal sedative effect is present.

The degree of sedation, based on HHAG, was the same with both α_2 -adrenergic agonists from 20 minutes after bolus administration to the end of CRI. However, based on reaction to acoustic stimulation by metallic noise, the horses might be more arousable with xylazine compared to romifidine. Sedation with α_2 -adrenergic agonists decreases the reaction to visual, acoustic and tactile stimulation. However, response is not completely abolished and intensity might be dependent on the individual horse.

Cardiovascular depression was more severe and prolonged with romifidine. Most of the cardiovascular effects were most pronounced during loading dose administration and tended to stabilize during CRI. Therefore, CRI is likely to be safer than repeated boli administration for prolonged sedation of horses using xylazine or romifidine.

Romifidine seems to provide more reliable sedation with a tendency to less ataxia, however in the cardiovascularly unstable patient xylazine might be safer.

In the present study, butorphanol did not reduce the α_2 -adrenergic agonist requirements in unstimulated experimental horses. The combination of xylazine with butorphanol at the doses used in the present studies cannot be recommended due to dangerous postural instability and inconsistent sedation.

4.3. Future studies

The main limitation of the studies regarding extrapolation to clinical practice is that we used healthy pain-free experimental horses. Under experimental conditions it is very difficult to mimic a clinical situation. Therefore, controlled clinical studies are inevitable. Studies comparing the xylazine and romifidine protocol under clinical conditions would be very interesting and also the role of butorphanol has to be further elucidated.

The developed xylazine-butorphanol protocol cannot be recommended due to the dangerous ataxia and early awakening observed. Possibly a lower loading dose of xylazine followed by a higher dose CRI would have provided a safer sedation protocol in combination with butorphanol. Further experiments are needed to develop such a protocol and to test for constant sedation and plasma concentrations.

Also the effect of the diuresis on electrolytes, glucose and cardiovascular function should be further studied and quantified.

5. Reference List

- Abrahamsen EJ (2007) How to: constant rate infusion for standing surgery restraint and analgesia. Proceedings BEVA Congress, Edinburgh, UK.
- Aguiar AJA, Medeiros LQ, Dessen MR et al. (2009) Hemodynamic and sedative effects of constant rate infusions of detomidine associated with butorphanol, and detomidine alone in conscious horses. Proceedings of the 10th World Congress of Veterinary Anaesthesia, Glasgow, UK, pp 169.
- Bettschart-Wolfensberger R, Clarke KW, Vainio O et al. (1999a) Pharmacokinetics of medetomidine in ponies and elaboration of a medetomidine infusion regime which provides a constant level of sedation. *Res Vet Sci* 67, 41-46.
- Bettschart-Wolfensberger R, Bettschart RW, Vainio O et al. (1999b) Cardiopulmonary effects of a two hour medetomidine infusion and its antagonism by atipamezole in horses and ponies. *J vet Anaesth* 26, 8-12.
- Bettschart-Wolfensberger R, Freeman SL, Bowen IM et al (2005) Cardiopulmonary effects and pharmacokinetics of i.v. dexmedetomidine in ponies. *Equine Vet J* 37, 60-64.
- Bidwell LA, Bramlage LR, Rood WA (2007) Equine perioperative fatalities associated with general anaesthesia at a private practice--a retrospective case series. *Vet Anaesth Analg* 34, 23-30.
- Brodbelt DC, Blissitt KJ, Hammond RA et al. (2008) The risk of death: the confidential enquiry into perioperative small animal fatalities. *Vet Anaesth Analg* 35, 365-373.
- Browning AP, Collins JA (1994) Sedation of horses with romifidine and butorphanol. *Vet Rec* 134, 90-91.
- Brunson DB, Majors LJ (1987) Comparative analgesia of xylazine, xylazine/morphine, xylazine/butorphanol, and xylazine/nalbuphine in the horse, using dental dolorimetry. *Am J Vet Res* 48, 1087-1091.
- Bryant CE, England GC, Clarke KW (1991) Comparison of the sedative effects of medetomidine and xylazine in horses. *Vet Rec* 129, 421-423.
- Bryant CE, Clarke KW, Thompson J (1996) Cardiopulmonary effects of medetomidine in sheep and in ponies. *Res Vet Sci* 60, 267-271.
- Bryant CE, Thompson J, Clarke KW (1998) Characterisation of the cardiovascular pharmacology of medetomidine in the horse and sheep. *Res Vet Sci* 65, 149-154.
- Bueno AC, Cornick-Seahorn J, Seahorn TL et al. (1999) Cardiopulmonary and sedative effects of intravenous administration of low doses of medetomidine and xylazine to adult horses. *Am J Vet Res* 60, 1371-1376.

- Clarke KW, Paton BS (1988) Combined use of detomidine with opiates in the horse. *Equine Vet J* 20, 331-334.
- Clarke KW, England GCW, Goossens L (1991) Sedative and cardiovascular effects of romifidine, alone and in combination with butorphanol, in the horse. *J Vet Anaesth* 18, 25-29.
- Corletto F, Rasis AA, Brearley JC (2005) Comparison of morphine and butorphanol as pre-anaesthetic agents in combination with romifidine for field castration in ponies. *Vet Anaesth Analg* 32, 16-22.
- Daunt DA, Dunlop CI, Chapman PL et al. (1993) Cardiopulmonary and behavioral responses to computer-driven infusion of detomidine in standing horses. *Am J Vet Res* 54, 2075-2082.
- DeRossi R, Jorge TP, Ossuna MR et al. (2009) Sedation and Pain Management with Intravenous Romifidine-Butorphanol in Standing Horses. *J Equine Vet Sci* 29, 75-81.
- Dyson DH, Pascoe PJ, Viel L et al (1987) Comparison of detomidine hydrochloride, xylazine, and xylazine plus morphine in horses: a double blind study. *J Equine Vet Sci* 7, 211-215.
- Ebert TJ, Hall JE, Barney JA et al. (2000) The effects of increasing plasma concentrations of dexmedetomidine in humans. *Anesthesiology* 93, 382-394.
- England GC, Clarke KW, Goossens L (1992) A comparison of the sedative effects of three alpha 2-adrenoceptor agonists (romifidine, detomidine and xylazine) in the horse. *J Vet Pharmacol Ther* 15, 194-201.
- England GC, Clarke KW (1996) Alpha₂adrenoreceptor agonists in the horse—a review. *Br Vet J* 152, 641-657.
- Figueiredo JP, Muir WW, Smith J et al. (2005) Sedative and Analgesic Effects of Romifidine in Horses. *Intern J Appl Res Vet Med* 3, 249-258.
- Freeman SL, England GC (1999) Comparison of sedative effects of romifidine following intravenous, intramuscular, and sublingual administration to horses. *Am J Vet Res* 60, 954-959.
- Freeman SL, Bowen IM, Bettschart-Wolfensberger R et al. (2000) Cardiopulmonary effects of romifidine and detomidine used as premedicants for ketamine/halothane anaesthesia in ponies. *Vet Rec* 147(19), 535-9.
- Freeman SL, England GC (2000) Investigation of romifidine and detomidine for the clinical sedation of horses. *Vet Rec* 147, 507-511.

- Freeman SL, Bowen IM, Bettschart-Wolfensberger R et al. (2002) Cardiovascular effects of romifidine in the standing horse. *Res Vet Sci* 72, 123-129.
- Gasthuys F, De Moor A, Parmentier D (1990) Haemodynamic changes during sedation in ponies. *Vet Res Commun* 14, 309-327.
- Gibbs N, Rodoreda P (2005) Anaesthetic mortality rates in Western Australia 1980-2002. *Anaesth Intensive Care* 33, 616-622.
- Gornemann T, von Wenckstern H, Kleuser B et al. (2007). Characterization of the postjunctional alpha 2C-adrenoceptor mediating vasoconstriction to UK14304 in porcine pulmonary veins. *Br J Pharmacol* 151, 186-194.
- Greene SA, Thurmon JC (1988) Xylazine--a review of its pharmacology and use in veterinary medicine. *J Vet Pharmacol Ther* 11, 295-313.
- Hamm D, Turchi P, Jochle W (1995) Sedative and analgesic effects of detomidine and romifidine in horses. *Vet Rec* 136, 324-327.
- Holopherne D, Faucher C, Desfontis J-C et al. (2005) Comparison of three sedative protocols for use in the horse: romifidine vs romifidine/morphine vs romifidine/butorphanol. *Proceedings of the AVA fall meeting, Newmarket, UK*, pp 52.
- Johnston GM, Eastment JK, Wood JLN et al. (2002) The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of Phases 1 and 2. *Vet Anaesth Analg* 29, 159-170.
- Johnston GM, Eastment JK, Taylor PM et al. (2004) Is isoflurane safer than halothane in equine anaesthesia? Results from a prospective multicentre randomised controlled trial. *Equine Vet J* 36, 64-71.
- Kalpravidh M, Lumb WV, Wright M et al. (1984) Analgesic effects of butorphanol in horses: dose-response studies. *Am J Vet Res* 45, 211-216.
- Katila T, Oijala M (1988) The effect of detomidine (Domosedan) on the maintenance of equine pregnancy and foetal development: ten cases. *Equine Vet J* 20, 323-326.
- Kerr CL, McDonnell WN, Young SS (1996) A comparison of romifidine and xylazine when used with diazepam/ketamine for short duration anesthesia in the horse. *Can Vet J* 37, 601-609.
- Kohler I, Armbruster S, Lanz F et al. (2004) Analgetische Wirkung von Romifidin kombiniert mit Butorphanol oder Levomethadon beim Pferd. *Tierärztl Prax* 32, 345-349.
- Kollias-Baker CA, Court MH, Williams LL (1993) Influence of yohimbine and tolazoline on the cardiovascular, respiratory, and sedative effects of xylazine in the horse. *J Vet Pharmacol Ther* 16, 350-358.

- Kruluc P, Nemec A (2006) Electroencephalographic and electromyographic changes during the use of detomidine and detomidine-butorphanol combination in standing horses. *Acta Vet Hung* 54, 35-42.
- Lavoie JP, Pascoe JR, Kurpershoek CJ (1992) Effect of head and neck position on respiratory mechanics in horses sedated with xylazine. *Am J Vet Res* 53, 1652-1657.
- Love EJ, Taylor PM, Murrell J et al. (2011) Assessment of the sedative effects of buprenorphine administered with 10 µg/kg detomidine in horses. *Vet Rec* 9, 168(14), 379.
- Luukkanen L, Katila T, Koskinen E (1997) Some effects of multiple administration of detomidine during the last trimester of equine pregnancy. *Equine Vet J* 29, 400-402.
- Marntell S, Nyman G (1996) Effects of additional premedication on romifidine and ketamine anaesthesia in horses. *Acta Vet Scand* 37, 315-325.
- Marntell S, Nyman G, Funkquist P, et al (2005) Effects of acepromazine on pulmonary gas exchange and circulation during sedation and dissociative anaesthesia in horses. *Vet Anaesth Analg* 32, 83-93.
- Miksa IR, Cummings MR, Poppenga RH (2005) Determination of acepromazine, ketamine, medetomidine, and xylazine in serum: multi-residue screening by liquid chromatography-mass spectrometry. *J Anal Toxicol* 29, 544-551.
- Moens Y, Lanz F, Doherr MG et al. (2003) A comparison of the antinociceptive effects of xylazine, detomidine and romifidine on experimental pain in horses. *Vet Anaesth Analg* 30, 183-190.
- Muir WW, Skarda RT, Sheehan WC (1979a) Hemodynamic and respiratory effects of xylazine-morphine sulfate in horses. *Am J Vet Res* 40, 1417-1420.
- Muir WW, Skarda RT, Sheehan W (1979b) Hemodynamic and respiratory effects of a xylazine-acetylpromazine drug combination in horses. *Am J Vet Res* 40, 1518-1522.
- Murrell JC, Hellebrekers LJ (2005) Medetomidine and dexmedetomidine: a review of cardiovascular effects and antinociceptive properties in the dog. *Vet Anaesth Analg* 32, 117-127.
- Nannarone S, Gialletti R, Veschini I et al. (2007) The use of alpha-2 agonists in the equine practice: comparison between three molecules. *Vet Res Commun* 31 Suppl 1, 309-312.
- Nannarone S, Sommer L, Cercione M et al. (2011) Romifidine or detomidine continuous infusion for standing elective surgery in 78 horses. *Proceedings of the Association of Veterinary Anaesthetists (AVA) Spring Meeting, Bari, Italy*, pp 133.

- Nilsfors L, Kvart C, Kallings P, et al (1988) Cardiorespiratory and sedative effects of a combination of acepromazine, xylazine and methadone in the horse. *Equine Vet J* 20, 364-367.
- Nolan AM, Besley W, Reid J et al. (1994) The effects of butorphanol on locomotor activity in ponies: a preliminary study. *J Vet Pharmacol Ther* 17, 323-326.
- Nyman G, Marntell S, Edner A, et al (2009) Effect of sedation with detomidine and butorphanol on pulmonary gas exchange in the horse. *Acta Vet Scand* 51, 22.
- Paton BS, Clarke KW (1986) A preliminary trial of detomidine/opiate combination in the horse. *J Assoc Vet Anaesth* 14, 44-52.
- Ringer SK, Portier KG, Fourel I et al. (2012a) Development of a romifidine constant rate infusion with or without butorphanol for standing sedation of horses. *Vet Anaesth Analg* 39, 12-20.
- Ringer SK, Portier KG, Fourel I et al. (2012b) Development of a xylazine constant rate infusion with or without butorphanol for standing sedation of horses. *Vet Anaesth Analg* 39, 1-11.
- Robertson JT, Muir WW, Sams R (1981) Cardiopulmonary effects of butorphanol tartrate in horses. *Am J Vet Res* 42, 41-44.
- Robertson JT, Muir WW (1983) A new analgesic drug combination in the horse. *Am J Vet Res* 44, 1667-1669.
- Rohrbach H, Korpivaara T, Schatzmann U, et al (2009) Comparison of the effects of the alpha-2 agonists detomidine, romifidine and xylazine on nociceptive withdrawal reflex and temporal summation in horses. *Vet Anaesth Analg* 36, 384-395.
- Romagnoli N, Guazzieri A, Ricciardi G et al. (2009) Romifidine levels in plasma and red blood cells after intravenous administration in horses: preliminary study. *Proceedings of the 10th World Congress of Veterinary Anaesthesia*, Glasgow, UK, pp 169.
- Rutkowski JA, Eades SC, Moore JN (1991) Effects of xylazine butorphanol on cecal arterial blood flow, cecal mechanical activity, and systemic hemodynamics in horses. *Am J Vet Res* 52, 1153-1158.
- Schatzman U, Armbruster S, Stucki F et al. (2001) Analgesic effect of butorphanol and levomethadone in detomidine sedated horses. *J Vet Med A Physiol Pathol Clin Med* 48, 337-342.
- Sellon DC, Monroe VL, Roberts MC et al. (2001) Pharmacokinetics and adverse effects of butorphanol administered by single intravenous injection or continuous intravenous infusion in horses. *Am J Vet Res* 62, 183-189.

- Sellon DC, Roberts MC, Blikslager AT et al. (2004) Effects of continuous rate intravenous infusion of butorphanol on physiologic and outcome variables in horses after celiotomy. *J Vet Intern Med* 18, 555-563.
- Solano AM, Valverde A, Desrochers A et al. (2009) Behavioural and cardiorespiratory effects of a constant rate infusion of medetomidine and morphine for sedation during standing laparoscopy in horses. *Equine Vet J* 41, 153-159.
- Stoelting RK, Hillier SC (2006) Pharmacokinetics and Pharmacodynamics of Injected and Inhaled Drugs. In: *Pharmacology & Physiology in Anesthetic Practice*. (4th edn). Stoelting RK, Hillier SC (eds). Lippincott Williams & Wilkins, Philadelphia, USA, pp. 3-41.
- Taittonen MT, Kirvela OA, Aantaa R et al. (1997). Effect of clonidine and dexmedetomidine premedication on perioperative oxygen consumption and haemodynamic state. *Br J Anaesth* 78, 400-406.
- Talukder MH, Hikasa Y (2009) Diuretic effects of medetomidine compared to xylazine in healthy dogs. *Can J Vet Res* 73, 224-236.
- Taylor PM, Browning AP, Harris CP (1988) Detomidine-butorphanol sedation in equine clinical practice. *Vet Rec* 123, 388-390.
- van Dijk P, Lankveld DP, Rijkenhuizen AB et al. (2003) Hormonal, metabolic and physiological effects of laparoscopic surgery using a detomidine-buprenorphine combination in standing horses. *Vet Anaesth Analg* 30(2), 72-80.
- Wagner AE, Muir WW, 3rd, Hinchcliff KW (1991) Cardiovascular effects of xylazine and detomidine in horses. *Am J Vet Res* 52, 651-657.
- Wertz EM, Dunlop CI, Wagner AE et al. (1994) Three chemical restraint techniques for mares undergoing standing colpotomy surgery. *Proceedings of the 5th International Congress of Veterinary Anesthesia*, Guelph, Canada. pp 133.
- Wilson DV, Bohart GV, Evans AT, et al. (2002) Retrospective analysis of detomidine infusion for standing chemical restraint in 51 horses. *Vet Anaesth Analg* 29, 54-57.
- Yamashita K, Tsubakishita S, Futaok S et al. (2000) Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *J Vet Med Sci* 62, 1025-1032.

7. Curriculum vitae and list of publications

7.1. Curriculum vitae

Curriculum vitae

Simone K. Ringer, Dr. med. vet., Dip ECVAA

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Education

1995 - 2000	Degree in Veterinary medicine, Universidad Complutense de Madrid (Spain)
1991 - 1995	High school with Swiss matriculation Type C1, Swiss College in Madrid (Spain)

Postgraduate education

Since Mai 2011	Senior lecturer in the Anaesthesiology Section, Vetsuisse Faculty, University of Zurich (Switzerland)
Since February 2008	PhD student at the Graduate School for Cellular and Biomedical Sciences, University of Bern (Switzerland)
2008 – 2009	Visiting researcher at the Equine Department, VetAgro Sup, Veterinary Campus of Lyon (France)

December 2009	Diploma of the European College of Veterinary Anaesthesia and Analgesia (ECVAA)
2006 – 2008	Lecturer in the Anaesthesiology Section, Vetsuisse Faculty, University of Zurich (Switzerland)
2003 – 2006	Resident in the Anaesthesiology Section, Vetsuisse Faculty, University of Zurich (Switzerland)
2005	Doctor in Veterinary Medicine (Dr. med. vet.), Vetsuisse Faculty, University of Zurich (Switzerland)
August 2003	Visiting veterinarian (4 weeks) of the Anaesthesia Department of the University of Florida, Gainesville (USA)
2002 – 2005	Doctoral Thesis (Dr. med. vet.), “Effects of extracorporeal shock wave therapy on sound equine bone and bone-tendon junction” Vetsuisse Faculty, University of Zurich (Switzerland)
2002 – 2003	Assistant veterinarian in the Equine Surgery Section, Vetsuisse Faculty, University of Zurich (Switzerland)
July 2001 – 2002	Visiting veterinarian in the Equine Surgery Section, Vetsuisse Faculty, University of Zurich (Switzerland)
October 2000 – Mai 2001	Assistance in a private equine clinic, HORSEPITAL, Madrid (Spain)

Responsibilities at the VSF Zurich beside anaesthesia service in large and small animals

Emergency Service in Anaesthesia (all species) and Critical Care (small animal)

Training of residents and interns

Classroom teaching and practical education of veterinary students

Supervision of doctoral candidates (Dr. med. vet.) and Master students

Languages

German and Spanish – bilingual

English – spoken fluently and well written

French – spoken and written

7.2 List of publications

Scopus link: <http://www.scopus.com/authid/detail.url?authorId=9276983400>

Original publications in peer-reviewed journals

First author:

1. Ringer SK, Portier K, Torgerson PR, Castagno R, Bettschart-Wolfensberger R. Evaluation of the degree of ataxia and the response to auditory, visual and tactile stimulation during loading doses followed by constant rate infusions (CRI) of xylazine and romifidine in horses, *accepted Vet Anaesth Analg*.
2. S.K. Ringer, C.C. Schwarzwald, K. Portier, A. Ritter, R. Bettschart-Wolfensberger. Effects on cardiovascular function and oxygen delivery of romifidine and xylazine loading doses followed by constant rate infusion (CRI), *The Vet J* 2012, 10.1016/j.tvjl.2012.06.036.
3. Ringer SK, Portier K, Fourel I, Bettschart-Wolfensberger R. Development of a romifidine constant rate infusion with or without butorphanol for standing sedation of horses. *Vet Anaesth Analg* 2012 Jan; 39: 12-20.
4. Ringer SK, Portier K, Fourel I, Bettschart-Wolfensberger R. Development of a xylazine constant rate infusion with or without butorphanol for standing sedation of horses. *Vet Anaesth Analg* 2012 Jan; 39: 1-11.
5. Ringer SK, Kalchofner K, Boller J, Fürst A, Bettschart-Wolfensberger R. A clinical comparison of two anaesthetic protocols using lidocaine or medetomidine in horses. *Vet Anaesth Analg* 2007 Jul; 34(4): 257-268.
6. SK Ringer, CJ Lischer, G Ueltschi. Assessment of scintigraphic and thermographic changes after focused extracorporeal shock wave therapy on the origin of the suspensory ligament and the fourth metatarsal bone in horses without lameness. *Am J Vet Res* 2005; 66(10): 1836-1842.

Second author:

7. K. Benredouane, S.K. Ringer, I. Fourel, O.M. Lepage, K.G. Portier, R. Bettschart-Wolfensberger. Comparison of xylazine-butorphanol and xylazine-morphine-ketamine infusions in horses undergoing a standing surgery. *Vet Rec* 2011; 169(14): 364.
8. M. Paula Larenza, Simone K. Ringer, Annette P.N Kutter, Aude Conrot, Regula Theurillat, Martin Kummer, Wolfgang Thormann, Regula Bettschart-Wolfensberger. Evaluation of anaesthesia recovery quality after low-dose racemic or S-ketamine

infusions during anesthesia with isoflurane in horses. *Am J Vet Res* 2009; 70(6): 710-718.

9. K Nuss, S Ringer, SW Meyer, B Schade, S Olerth. Shoulder lameness caused by the inflammation of the subtendinous bursa of the infraspinatus muscle in three cows. *Vet Rec* 2007; 160: 198-200.
10. Lischer CJ, Ringer SK, Schnewlin M, Imboden I, Fürst A, Stockli M, Auer J. Treatment of chronic proximal suspensory desmitis in horses using focused electrohydraulic shockwave therapy. *Schweiz Arch Tierheilkd* 2006; 148(10): 561-568.
11. Bischofberger AS, Ringer SK, Geyer H, Imboden I, Uletschi G, Lischer CJ. Histomorphologic evaluation of extracorporeal shock wave therapy of the fourth metatarsal bone and the origin of the suspensory ligament in horses without lameness. *Am J Vet Res* 2006; 67(4): 577-582.
12. K Kalchofner, S Ringer, J Boller, K Neges, SBR Kaestner, C Lischer, R Bettschart-Wolfensberger. Clinical assessment of anaesthesia with isoflurane and medetomidine in 300 equidae. *Pferdeheilkunde* 2006; 22(3): 301-308.

Last author:

13. Mauch J, Weiss M, Spielmann N, Ringer SK. Electrocardiographic and blood pressure alterations caused by intravenous injection of ropivacaine – a study in piglets, *accepted Pediatric Anesthesia*.
14. R. Bettschart-Wolfensberger, S. Dicht, C. Vullo, A. Frotzler, J. Kümmerle, S. Ringer. A clinical study on the effect in horses during medetomidine-isoflurane anaesthesia, of butorphanol constant rate infusion on isoflurane requirements, cardiopulmonary function and on recovery characteristics. *Vet Anaesth Analg* 2011; 38: 186-194.

Co-author:

15. Schefer DS, Hagen R, Ringer SK, Schwarzwald CC. Laboratory, Electrocardiographic, and Echocardiographic Detection of Myocardial Damage and Dysfunction in an Arabian mare with Nutritional Masseter Myodegeneration. *J Vet Intern Med* 2011; 25(5): 1171–1180.
16. Picek S, Kalchofner KS, Ringer SK, Kummer M, Fürst A, Bettschart-Wolfensberger R. Anaesthetic management for hydropool recovery in 50 horses. *Pferdeheilkunde* 2010; 26(4): 515-522.

17. Kalchofner KS, Picek S, Ringer SK, Jackson M, Haessig M, Bettschart-Wolfensberger R. A study of cardiovascular function under controlled and spontaneous ventilation in isoflurane-medetomidine anaesthetised horses. *Vet Anaesth Analg* 2009; 36: 426-435.
18. M Boller, A Fürst, S Ringer, R Bettschart-Wolfensberger. Complete recovery from long standing priapism in a stallion after propionylpromazine/xylazine sedation. *Equine Vet Educ* 2005; 17(6): 305-311.

Book chapters

1. Ringer SK: Chemical Restraint for Standing Surgery. In: Jörg A. Auer and John A. Stick (eds): *Equine Surgery*, 4th edition (2011). Elsevier Saunders, 253-262.

Online editorials/reviews:

1. Alpha₂-adrenergic agonists constant rate infusions (CRI) for prolonged standing sedation of horses. *Review Association of Veterinary Anaesthetists (AVA) online newsletter*, January 2012.

Abstracts/Conference Proceedings

1. S.K. Ringer, C.C. Schwarzwald, K. Portier, A. Ritter, R. Bettschart-Wolfensberger. Effects on cardiovascular function and oxygen delivery of romifidine and xylazine loading doses followed by constant rate infusion (CRI) in standing horses. Association of Veterinary Anaesthetists Spring Meeting April 2011, Bari, Italy.
2. S.K. Ringer, C.C. Schwarzwald, K. Portier, A. Ritter, R. Bettschart-Wolfensberger. Blood glucose, acid-base and electrolyte changes during loading doses of romifidine and xylazine followed by constant rate infusion (CRI) in horses. Association of Veterinary Anaesthetists Spring Meeting April 2011, Bari, Italy.
3. Schefer KD, Hagen R, Ringer SK, Schwarzwald CC. Masseter myodegeneration and myocardial damage due to selenium deficiency. 4th ECEIM congress February 2011, Hannover, Germany.
4. S.K. Ringer, K. Portier, P.R. Torgerson, R. Castagno, R. Bettschart-Wolfensberger. Evaluation of the degree of ataxia and the response to auditory, visual and tactile stimulation during constant rate infusion (CRI) of xylazine and romifidine in horses. Association of Veterinary Anaesthetists Spring Meeting March 2010, Cambridge, UK.

5. K.S. Kalchhofner, J. Werner, S.K. Ringer, T.G. Guerrero, M. Hässig, R. Bettschart-Wolfensberger. Comparison of 'whoosh' test and epidural pressure waves measurement for identification of the epidural space in dogs. Association of Veterinary Anaesthetists Spring Meeting March 2010, Cambridge, UK.
6. R. Bettschart-Wolfensberger, S. Dicht, C. Vullo, A. Frotzler, J. Kümmerle, S. Ringer. Isoflurane in combination with a CRI of medetomidine-butorphanol or medetomidine in horses. Association of Veterinary Anaesthetists Spring Meeting March 2010, Cambridge, UK.
7. K.S. Kalchhofner, M. Schweizer-Kölliker, S.K. Ringer, T.G. Guerrero, R. Bettschart-Wolfensberger. Incidence of delayed hair growth and pruritus after epidural anaesthesia in dogs. 10th World Congress of Veterinary Anaesthesia 2009, Glasgow, UK.
8. Ringer SK, Portier K, Gaudefroy T, Fourel I, Bettschart-Wolfensberger R. Development of a xylazine constant rate infusion (with or without butorphanol) for standing sedation of horses. Association of Veterinary Anaesthetists Spring Meeting 2009, Helsinki, Finland.
9. Ringer SK, Portier K, Hébert P, Fourel I, Bettschart-Wolfensberger R. Development of a romifidine constant rate infusion (with or without butorphanol) for standing sedation of horses. Association of Veterinary Anaesthetists Spring Meeting 2009, Helsinki, Finland.
10. Ringer SK, Portier K, Gaudefroy T, Fourel I, Bettschart-Wolfensberger R. Development of new protocols for sedation of standing horses – Pilot studies. 3rd Graduate School Student's Symposium 2009, Bern, Switzerland.
11. M.P. Larenza, K. Kluge, A. Conrot, A. Kutter, S. Ringer, M. Jackson, R. Bettschart. Cardiovascular effects and recovery quality after S-ketamine or medetomidine infusions supplemental to isoflurane anaesthesia in horses. Association of Veterinary Anaesthetists Autumn Meeting 2008, Barcelona, Spain.
12. Olga Martin Jurado, Simone Ringer, Jean-Michel Hatt, Regula Bettschart-Wolfensberger. Revision de los casos clinicos de especies exoticas monitorizados con el indice bispectral (BIS). Congreso Sociedad Espaniola de Anestesia y Analgesia Veterinaria (SEAAV) 2008, Barcelona, Spain.
13. Bettschart R., Picek St., Ringer S., Kalchhofner K., and Furst A. Retrospective report of anesthesia management before and during hydropool recovery in 50 horses. 14th

International Veterinary Emergency and Critical Care Symposium (IVECC) 2008, Phoenix, USA.

14. Nuss K, Ringer S, Schade B, Olerth S, Meyer S. Shoulder lameness caused by infection of the subtendinous bursa of the infraspinatus muscle in 5 cows. 24th World Buiatrics Congress 2006, Nice, France.
15. SW Meyer, S Ringer, B Schade, S Olerth, K Nuss. Shoulder lameness caused by the inflammation of the subtendinous bursa of the infraspinatus muscle in three cows. BUIATRISIMA 2005, Bern, Switzerland.
16. S. Ringer, K. Kalchofner, J. Boller, A. Fürst, R. Bettschart-Wolfensberger. Comparison of lidocaine versus medetomidine constant rate infusion during isoflurane anaesthesia in horses. Association of Veterinary Anaesthetists Spring Meeting 2005, Rimini, Italy.
17. K. Kalchofner, S. Ringer, J. Boller, K. Neges, S.B.R Kästner, C. Lischer, R. Bettschart-Wolfensberger. Clinical assessment of anaesthesia with isoflurane and medetomidine in 300 horses. Association of Veterinary Anaesthetists Spring Meeting 2005, Rimini, Italy.

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9. Declaration of originality

Declaration of Originality

Last name, first name: Ringer, Simone Katja

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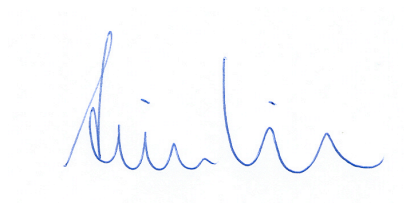
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I am aware that in case of non-compliance, the Senate is entitled to divest me of the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 20, of 17 December 1997.

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Simone Katja Ringer